THE

AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

CONTENTS

VOLUME TWENTY-ONE NUMBER 2

MARCH-APRIL

1955

The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

The American Journal of Medical Technology

VOLUME 21 MARCH-APRIL, 1955 NUMBER 2

MEDICAL TECHNOLOGY—EDUCATIONAL PROBLEMS*

By SISTER M. ALCUIN ARENS, M.T. (ASCP)†
Duluth, Minnesota

The curricula and education in medical technology are at the present time in a very primitive and chaotic state. The means of improving this situation appears to be in correcting the thinking of the public on three points. These points are, first, an understanding of the true meaning of medical technology; second, an acceptance of the division of labor and a hierarchy of personnel in medical technology, and third, a recognition of the fact that the process of education in medical technology must be based on the same pedagogical principles as is any system of education. The last point is the most important pertaining as it does to the position of the key person in medical technology, the medical technologist.

By some, from the very beginning, medical technology was regarded as a mechanical exercise not requiring much thinking. In the minds of these individuals it was a running of tests and doing some typewriting, or assisting the doctor in one way or another in his technical work. Some modernists sound no different than these in that they, too, regard medical technology as the skill derived from being drilled in not more than six to ten routine techniques.

On the other hand even in the earlier days keen observers regarded medical technology as an intricate, complex though essential technical phase of laboratory diagnosis.

If medical technology ever was a humdrum of procedures, more or less automatic, would sincere pathologists have commended the medical technologists as they did? In 1939 Dr. M. Bodansky in his own words pointed out this pertinent fact, "If the present trend continues it is inevitable that the future of the properly qualified technologist should . . . follow a higher level in regard to both professional and economic status." And then he documented his assertion with these additional words, "Evidence of

*Registry Award: Read before ASMT convention, June, 1954, Miami Beach, Florida.
†Deceased.

this is all around us . . . we now have persons with university degrees, with honors in bacteriology and chemistry, who are entering training in medical technology."

If medical technology were merely mechanical procedures, the Armed Forces would never have directed maturer minds to try a hand at it. The undesirable features that drove away the recruits mentioned below were, in the words of Miss Cora Louise Miller, not the nature of the work but the hours and the pay. This evidence Miss Miller gave following World War II. These are her words:

> I have had quite a lot of experience in training G.I.'s, W.A.C.'s, and a few civilians. In my 3½ years experience in the Army Hospital I have had a chance to expose about 100 men and women to a smattering of Laboratory Technology. Most have not liked it, mainly because of the long hours in the laboratory and "Emergency" call at night. Four have been excellent technicians but have had like training before coming into the Army. One was a teacher of Bacteriology in a College, and was also given additional training at Walter Reed. He was given a Commission by the President and assigned to the Malarial Control program. Another was a dye chemist in a textile factory. He was also Walter Reed trained. A third was a mortician. He made an excellent technician. If Laboratory Technology paid better, he would probably be in it, but instead he is back at work with his father in the Undertaking Business. The fourth is a W.A.C. She has had some lecture training in clinical work after discharge from the Army, but she wanted to study Social Law, not technology.

An insight into the very scope of the work conveys the idea that medical technology has never been anything simple. This is equally true from the early days when laboratory examinations first became acceptable medical practices to the present time when these examinations have become indispensable. The routine of the laboratories of the twenties was similar to the present routine although comprising only a fraction of its volume. This is the same as saying that the routine of the second decade differs from the routine of the mid-twentieth century in that there is a "change from the personal service of the Pathologist to a few" to the "mass production service to all." There is a change from a meager use of chemistry to mass application of chemical principles and practices, in keeping with the progress of science. Medical Technology has gone from a little volume of what was sound and applied science of the 20's to staggering loads of what is sound and applied science of the 50's. Then in addition to these changes in routine, concomitant with advances in research, there are additional responsibilities taken on by an increasing number of laboratories, introducing extensive education programs for

medical men and the personnel of allied fields in medicine. And furthermore, though the records say little about it, there must have been work of a research nature going on in many clinical laboratories then as now. For if research had always been limited only to a few specialized centers surely medicine would not have made the advances it has.

It remained for Dr. Walter S. Simpson, one-time president of the American Society of Clinical Pathologists, to point out that this work once considered important enough for clinical pathologists to perform must, because of the increase in volume, be done by scientists whose specific preparation enables them to carry on where the pathologist left off. His words are these, "The ever-growing number of laboratory procedures and the time and skill demanded for the performance of many of them had made it necessary for the clinical pathologist to delegate much of the work to lay technicians."

The work the pathologist had delegated to the "lay technician" is then for the greater part this routine, this research, and these educational responsibilities, the composite of which constitutes medical technology. This is the intricate, complex and essential, though technical, phase of laboratory diagnosis (and of medical prognosis and therapeutics). It is the ultimate meaning the public must apply to medical technology. Dr. Simpson's "lay technician" is the medical technologist, the scientist, who perforce, is expected to carry on in the same scientific manner in which the pathologist did. However, when the public refers to the performance of a limited number of tests as medical technology, and the performer as a technologist, obviously it is in error. A limited number of tests is typical of, but constitutes merely a process, or a useful tool in medical technology. The performer thus limited is but a laboratory assistant.

The concept of medical technology as an intricate whole with constituent processes, some capable of being performed by assistants, leads to a grasp of the next point, the division of labor and the gradation of laborers in medical technology. A possibility of a division of labor and the hierarchy of laborers in medical technology was already foreseen by the Secretary of the original Board of Registry and some of his colleagues. The plan for a hierarchy appeared in the rough draft of the Constitution and By-Laws of the Registry written prior to 1928. At this time three types of laboratory workers were proposed. This plan was not adopted for reasons explained by Mrs. Anna R. Scott, the Board's first registrar, and Dr. Kano Ikeda, the Board's first secretary. Mrs. Scott said, " . . . we human beings are so constituted that there always arise contentions and petty differences, blocs form and one faction or another defeats or delays the best plans." Dr. Kano Ikeda spoke similarly but much later in these words, "It

amuses me to recall how stubbornly and consistently, and sometimes even underhandedly, some of the standpatters fought . . . ideas . . . which in spite of their vigorous objections, have been and are being gradually accepted and put into practice."

In 1928 the Registry settled for two classes and made what proved to be a paper provision for specialists. "Perhaps the most difficult task which confronted the first Committee on Registration was the question of how to classify the laboratory workers," said Dr. Kano Ikeda after being secretary not only to the first Board but to subsequent boards for almost twenty-five consecutive years. He "perennially proposed several classes," and out of the first proposal of "laboratory assistant," "laboratory technician" and "medical technologist" only "laboratory technician" and "medical technologist" were adopted, but by 1936 these two had been merged into the one "medical technologist."

The settling for "medical technologist" solved a contemporary issue which is too lengthy to discuss here, but it set aside again the 1928 opinion that for the good of the medical technology, the profession should yield to a gradation of laborers. In 1948 another attempt was made to introduce classes of workers. The full description of the classification cannot be given here but six classes were proposed and given a trial. The reawakening of interest in 1948 did not produce the proper solution either. Over a period of five years, 1948-1953, only 180 histological technicians, 37 chemists, 21 bacteriologists, 4 specialists and 86 aides were certified in classes other than the familiar medical technologist of the 1936 definition. This is factual evidence that the hierarchy of laborers established in 1948 is not the answer either but it argues for a hierarchy of perhaps another type.

Thus the trials and errors of the past twenty-five years have never completely smothered the recurrent question, "Should the duties of the medical technologist be staggered according to complexity and performed by individuals with different degrees of preparedness, or is it a one-man job?" Has the five-year trial of the 1948 plan indicated the need for a change, in substance, to the initially proposed plan of the Board of Registry or to another? If it suggests the 1928 plan proposing three gradations, shall they be named as originally proposed "laboratory assistant," "laboratory technician," and "medical technologist"? It seems that a better substitute for "laboratory assistant" would be "laboratory aide"; that the "laboratory technician," since it is a misnomer, be abolished; and, "medical technologist" be retained, indicating as it does, the key person or general practitioner in medical technology. The third member, any one above the general practitioner would be a specialist, whether in research, education, or administration, and such a one be designated "medical technologist" but with further qualifications. The

Armed Forces designated those attached to the medical service by the insignia, using the caduceus for the basic symbol, and suitable letters on gold or silver to differentiate the doctor from the dentist, and the medical officer from the medical doctor. Could not the same system be adopted to designate the specialist in medical technology from the general practitioner?

To substitute "aide" for "assistant" is in keeping with the practice in the nursing profession, and categories the lesser helper as she truly is, an aid to, and not an equal of, the key person. For that reason it might be well to spell "aid" with an "e" thus "aide" as in military practice. The "laboratory aide" need have no certification, no insignia, but stand subject to service to the medical technologist in whatever capacity the staff chooses. Thus ranking from the lowest to the highest, the gradation is: "laboratory aide," "medical technologist, M.T. (ASCP)" and "medical technologist, M.T. (ASCP)" with some further insignia.

This brings the consideration to the third point, a recognition of the fact that the process of education in medical technology must be based on the same pedagogical principles as in any system of education. The thinking of the past quarter century about education in medical technology is as confused as was the thinking about the nature of medical technology. That second decade group considering medical technology something mechanical argued that the medical technology student should learn to do by doing, and the practical period of one full year was the answer. Among these 1920-ers was a smaller group that allowed economical factors to color their thinking. These argued that the student should not get more education than merely to learn to do as the pathologists show them or they would want more pay. Akin to this was the thinking of another group which held that the preparation of the student should be slight so as to make it possible for persons in modest circumstances to buy the service. Today a small number of modernists also contend that a drill of techniques constitutes the preparation. Other contemporaries imply that much consideration about the education is out of the question since there is nothing stabilizing in medical technology, many recruits ultimately changing careers to follow home making. They say extensive preparation for them is really a waste. This tenet is refuted in another paper by the author. So much for those regarding education in medical technology as a primitive rote process of learning.

On the other hand, in the twenties as now in the fifties there were proponents of a more satisfactory type of education for medical technologists. These advocates recognized that not only knowledge, but also a great deal of independent technical judgment, is required in the clinical laboratory. Even more, in the

modern hospital where a teaching program is being pursued for medical interns, residents, and diplomates of various branches of medicine, the medical technologist plays a part. The medical technologist will not satisfy these individuals any more than he can hope to satisfy the present generation of practitioners unless he is fully equipped to meet their demands on laboratory services. He must learn to think, to talk, and to act as they do, in all that pertains to medical technology without becoming a diagnostician or therapeutist. Hence the pathologists who do not regard medical technology as a mere automatic exercise admit that nothing less than a complete college education will satisfy as the preparation for it. Such a preparation exceeds even that of the average registered nurse and incidentally sets medical technology academically equal to or superior to nursing. Thus if the nurse spends three years in preparation, the medical technologist must be willing to spend the necessary four years to fit himself for the general practice of medical technology.

There is apparently no written record stating the objectives in education for the field of medical technology. For the greater part in the past it has been education without educators. Those having had a hand in the preparation of the medical technologists had it secondary to some other interest. Thus, there was the American College of Surgeons which about 1915 worked in the interest of hospital efficiency. Insofar as the clinical pathological laboratory played a part in that efficiency were they interested in seeing that employees in the laboratory were prepared who would promote the efficiency program. In the early twenties individual pathologists whose primary object was to advance the practice of pathology took an interest in preparing some assistants for themselves. Between 1915 and 1924 there were the bacteriologists and chemists who appeared to be championing the education of medical technologists but their primary object was to safeguard their own careers against the influx of quacks. Between 1923 and 1924 legal-minded medical men became interested but primarily to watch licensure of laboratories and laboratory helpers. Lastly, about the middle of the twenties, groups of pathologists and some physicians became interested, but primarily to safeguard the diagnosis, prognosis and treatment of disease by laboratory methods. Some of these pathologists formed the nucleus for that body conceived in 1928 known as the Registry of Medical Technologists, functioning under the auspices of the American Society of Clinical Pathologists.

But the Registry's object was not primarily nor wholly the education of medical technologists. Its primary object was then, and with one exception still is, to safeguard the patient by identifying the clinical laboratory worker through conducting a certifying agency for him; by identifying the "approved school," and by

enforcing the ethical practice of medical technology. In 1936 the Registry passed the responsibility of evaluating the competency of the "approved school" on to the Council of Medical Education and Hospitals of the American Medical Association.

In the absence of educators in medical technology it becomes evident why so much of the literature pertaining to medical technologists carries the passage "train the medical technologist," and not "educate the student." Prosaic as it may sound, elephants are trained, but rational human beings are educated. From all this, does it not become evident why the "approved school" is still in a chaotic and primitive state?

The blame for the current condition in the "approved school" is sometimes directed toward the Registry of Medical Technologists (ASCP) but is it justified? If properly interpreted, its words and acts indicate that all during the twenty-five years of its existence the Registry tried to adhere to its primary purpose of existence—to judge the competence of the medical technologist and of the school. When it gave up evaluating schools it continued to judge the competence of the medical technologist.

The Registry said on occasions that it is not an educational institution. Therefore, it never became empowered to grant degrees nor to administer a course of studies. The words of Mrs. Anna R. Scott, its first Registrar, are plain on this. She wrote in 1937, " . . . the Registry is not a college of learning in the strict sense . . ." In issuing certificates to individuals in recognition for profesional preparedness the Registrar said, "Your scientific status is attested by the educational, scientific and technical knowledge required for all those certified by the Registry." Another Registry statement written elsewhere bears the same interpretation, "(The Registry) is maintaining standards of educational and technical qualifications." When the Registry supported measures to screen out the naturally unfit applicant, it recognized in the act, a means of sustaining high professional standards. Its official words were clear on this. "(The Registry admits) attention to personal and psychological attributes (are) now recognized as not only desirable but necessary." Dr. Philip Hillkowitz, for many years a member of the Registry Board but now speaking unofficially reiterated the Board's stand by saying, "It was argued and with some justice that a college education was not necessarily superior to native ability and intelligence." These typical assertions emphasize the primary objectives of the Registry as a certifying agency, zealous to promote high standards educationally. Now to show it did the same by its actions.

Chronologically, the Registry defined professional standards for medical technology, did all in its power to enforce them and as time passed made the necessary adaptations to keep abreast of changes. In trying to define standards the Registry began where

the surgeons had left off. The American College of Surgeons, in its code for hospital efficiency, had formulated a clause that applied to the clinical pathological laboratory. In this clause the surgeons recognized certain fields of science as vital in building up the clinical laboratory's contribution to hospital efficiency. Among these were chemistry, clinical microscopy, bacteriology, serology and pathology. There is nothing to disprove that the pathologists borrowed these ideas from the surgeons, and using them for a core, formulated standards for the profession of medical technology, making the major services in medical technology include chemistry and the others named above. About this core were affixed the details for the standards of the profession, no doubt culled from data obtained from surveys made of medical technologists currently employed at the time of the inception of the Registry. Changes made in the years that followed were made not because of re-assessment of educational objectives for there were none, but presumably because of greater laboratory utility. In this wise the Board compiled and also maintained "essentials" in which that student personnel was to be prepared.

These "essentials" in the preparation of medical technologists included pre-technical courses of a scientific nature, first required at a high school level, later at the college level, which courses were to be administered by the college faculty. Technically, the Registry prescribed a period of practical work in a laboratory which was to be administered by the hospital staff under various types of directorship—part-time pathologist, full-time pathologist, and more recently a certified pathologist or his equivalent. The Registry evaluated transcripts of college credits presented by the applicants from "approved schools" for certification but this only in recent years. Taken as a composite, all these acts are not the duties of an institution of higher learning but of a certifying bureau.

Already as early as the thirties, even hasty critics recognized the Registry's primary intention of being a certifying agency. This admission is found in the following paragraph which incidentally airs the critic's views on education, educators and curricula in medical technology.

"Are there any requirements for becoming a medical technician?

"In truth, no. The promised boycott in a few years of all technicians who fail to register in the Registry of Technicians and subscribe to its requirements in order to work in a hospital approved by the A.M.A. is a threat. This is a minority move to coerce the technician and blind him so that he will not seek true standards. Some pathologists say given an orderly mind and average horse sense any girl can be trained to be a technician. Others say a background in the basic sciences and specific laboratory training is necessary.

"They must be taught not by . . . unqualified instructors but by persons who know how to teach and can provide . . . a solid foundation in the principles of chemistry, biology and physics upon which they can continually and successfully build up proficiency in their calling. These instructors are found in the universities."

Incidentally the statement that the Registry "boycott" was a threat is disproved, by statistics, national in scope. As of 1952, 77.7 per cent of the "approved schools" conducted Registry examinations and more than 88 per cent did not disprove them showing the popularity of the voluntary certifying agency. In a limited survey made in 1950 a little less than 59 per cent of the "teachers" were M.T.(ASCP) diplomates, and in a significant number all the "teachers' were Registry M.T.'s. This last statement is not to be misconstrued; it does not argue that diplomates without further preparation to teach are the teachers medical technologists should have in their student days, nor that the "approved school" is satisfactory as it now stands. It merely argues that we are working in the right direction.

This crisis in education in medical technology comes at a time when there is a general crisis in education, national in scope. The contributory factors to the general crisis are being studied. The American Council on Education has arranged for a nation-wide study of education as it pertains to women in the light of the impact of changing social conditions. Society in the 20th century has undergone change in the function of the family, school, government, and economic institutions. This change has affected women particularly. Women are being admitted to the fields of politics, economics and institutions of higher learning. The real dilemma of the century is the shortage of professional women workers, women in such groups as teachers, nurses, social workers, nutritionists, medical record librarians, and medical technologists. The national economic status has provoked an inflation which makes a double salary essential in many homes. In this distinctly twentieth century atmosphere a system of education must make provision for a livelihood and for the art of living.

What trend will education have to take to consider women as effective individuals, as gainfully engaged employees, as members of a democracy, and as "creators" and even "perpetuators of values"? Before World War I women were inconspicuous even in institutions of higher learning. Those who sought degrees sought them in arts, philosophy, science and literature. After World War I there was a marked development of technical courses and these were open to women. Now in the middle of the twentieth century, all fields of endeavor are open equally to men and women, in almost all institutions.

Another contributory feature to the present crisis in education is the inability of educators to decide on an education commensurate with the needs of the age and still keep it liberal or general. First of all what is understood by education? Education is sometimes defined as the formal means for supplying the members of society with common experiences intellectually. Then what is understood by general education? It is sometimes defined as the irreducible minimum of intellectual experiences vital for a person to live in society. Really general education is the unifying element of a culture. Between general education and liberal the difference is mainly one of degree. In America in the current century with equality of enterprise for both men and women these experiences must cover for all, the political, social, economical, psychological, and moral character of the age. America being a Christian democracy, these experiences must be predicated on the worth and the dignity of the human being. This, then, defines liberal education. Those upon whom the duty falls to make the decision of what system of education and what curriculum to pursue in medical technology should not all be found in the field of medicine. The chaos in education in medical technology is just one evidence to confirm this assertion. Surely academic administration of a program of education, in America, is a thing of the past. Academic interests and medical interests must be merged in the same program so that the whole individual is educated, not merely "trained." This decision, a mutual one on what constitutes a liberal education must be reached by both factions with poise, with self-confidence, and with the humility to begin anew, that medical technologists may be given their true position in the field of medicine, education and in society as a whole.

A PROGRAM OF REFRESHER TRAINING OF MEDICAL TECHNOLOGISTS IN GEORGIA*

By CAROLYN MARTIN, B.S., M.T. (ASCP)

Department of Biochemistry, Emory University, Univerity, Ga.

The Georgia Society of Medical Technologists is especially proud of the program of refresher courses which we have co-operated with the Georgia Department of Public Health in presenting during the past year. Many of us have long been interested in the improvement and standardization of laboratory procedures throughout the State, for we feel that this should be one of the ultimate goals of any state organization of technologists. We recognize the program of refresher training which we have attempted in the past year as a partial realization of a larger plan which we conceived some time ago and toward which we have worked through the years. We admit that this appears to represent meager accomplishment in tangible progress. As I trace briefly the evolution of this actuality from our original idea, I can not hope to have you understand our feeling of pride. In order really to understand, it would be necessary for you to have experienced our hopes and dreams on the one hand and our obstacles and disappointments on the other.

Our active efforts toward a standardization program date from a meeting of the Atlanta Society of Medical Technologists in February, 1951. Dr. George Lewis of the Department of Biochemistry of the Emory University School of Dentistry was the speaker at this meeting. Dr. Lewis was serving as consultant to the State of Florida in a program of consulation and evaluation, and he discussed the program in Florida with our group. This program was financed with funds from the state licensure fees. He urged the Georgia Society to attempt to initiate a similar program without resorting to state licensure for financial resources.

The Board of Directors of the Georgia Society met the following week-end, and the feasibility of attempting such a program was discussed. A Standardization Committee of three members was appointed to formulate plans. This committee met the following week and drafted a plan which was admittedly visionary. We dreamed a big dream, because we did not know how to dream a little one. Reactions to our plan on the part of pathologists with whom we later discussed it varied all the way from our being told that we had planned a program that would break the United States Treasury to our being accused of planning to set up independent laboratories. We realized that we had planned a program that was so extensive that nothing short of a miracle would make it possible for many years. We became so enthusiastic over our plans that we almost began expecting a miracle—until we began knocking on doors and

*Read before ASMT, June, 1954, Miami Beach, Florida.

spending discouraging afternoons in the best offices in Atlanta. We thought it best to plan the program in its entirety so that any beginning might be fitted into the whole.

It seemed logical to the Standardization Committee that the program be undertaken by the Georgia Society of Medical Technologists with the co-operation of the Georgia Association of Pathologists, the Medical Association of Georgia, and the Georgia Department of Public Health. These seemed to be the groups who should be responsible for such a program, and out of a combination of these groups we hoped to find the interest, knowledge, and financial backing necessary to operate such a program. We proposed to offer an evaluation and consultation service in biochemistry to every laboratory in the State of Georgia. The organization was to consist of a working committee of technologists proficient in the field of biochemistry, two representatives from the Medical Association, and one representative from the Georgia Department of Public Health, and an advisory committee consisting of two representatives from the Georgia Association of Pathologists. We planned for medical technologists to shoulder most of the burden, for we felt that this was primarily our problem and that we should take the lead in solving it. If medical technology is ever to come into its own, medical technologists must prove that they not only have the ability but the foresight and initiative to solve their own problems. It would be necessary to employ the services of a biochemist to act as consultant and to prepare the solutions for the evaluation study. Laboratories would be encouraged to participate on a voluntary basis through the influence of the Georgia Association of Pathologists and the Medical Association of Georgia and would pay a given fee for the services rendered. The working committee of technologists was to assist the biochemist with the consultation service. It was hoped that the Georgia Department of Public Health might make a substantial contribution in the form of using its facilities to mail out the evaluation study.

It seemed to the committee that if we could secure a commitment from the Georgia Department of Public Health to participate in the program to this extent, that we could more easily convince the Pathologists' Association and the State Medical Association of the practical value of the plan. The committee met with Dr. T. F. Sellers and Dr. E. J. Sunkes of the Georgia Department of Public Health in March. They received us graciously and were interested in the program to such an extent that Dr. Sellers offered to request a grant from the State of Georgia to defray the expenses of the entire program. This offer was, of course, far beyond our expectations. Some of us understood medical relationships well enough to raise the question of authority and, as was to be expected, the Georgia Department of Public Health would control the entire project if state funds financed it. Dr. Sellers emphasized the necessity of our having

the approval of the Georgia Association of Pathologists before any such program could be undertaken. Some of us were very dubious about accepting state participation to such an extent. Let me emphasize that relations between our organization and the Georgia Department of Public Health have always been of the most friendly nature. However, in formulating our plans we had been very specific in planning divided control among the groups concerned and were reluctant to turn over control to any one group even in exchange for the very vital financial backing. It was then deemed advisable to ask the advice of our ASCP Counselor. At this point many of us become increasingly aware of the intensely protective attitude of private medicine against any encroachment by any state agency.

Dr. Darrell Ayer of Atlanta, our ASCP Counselor, checked into the situation at a local and also a national level and advised us against participating in a program in which the state was involved to such an extent.

The most logical alternative was for the program to be undertaken as a joint project of the Georgia Association of Pathologists and the Georgia Society of Medical Technologists. The Georgia Association of Pathologists is badly handicapped by wide geographical distribution. The Atlanta Pathological Society was organized in April, 1951, to afford the Atlanta pathologists an organization which is not thus handicapped. This group, of course, comprizes a major portion of the Georgia Association of Pathologists. This group recognized the need for evaluation studies—both medical technologists and clinical pathologists. At the first meeting in June, 1951, a resolution was adopted empowering the Committee on Technical Methods and Standards to work out a program of standardization provided the program entailed co-operation between the Georgia Society of Medical Technologists and the Georgia Association of Pathologists and was not controlled even in part by any outside agency or individual. The Atlanta Pathological Society was fortunate in having Dr. F. William Sunderman, President of ASCP, serve as chairman of this committee. The Standardization Committee of GSMT met with Dr. Sunderman twice in June. It was hoped that the committees of the two organizations could sponsor an evaluation study at a local level at an early date as a pilot experiment for an evaluation at a state level at a later date. However, no further progress was made with these plans primarily because of the loss of key persons. Dr. Sunderman left Atlanta, and it was at about this time that Dr. George Lewis went to Florida as full-time director of the program in that state.

At our annual convention in Augusta in May, 1951, Dr. R. C. Williams of the Division of Hospital Services of the Georgia Department of Public Health spoke to the Georgia Society of Medical

Technologists, outlining to us the problem of staffing the Hill-Burton hospitals. I was elected president of GSMT at this meeting and appointed a special Hospital Services Committee to work with Dr. Williams. As an ex-officio member of the committee I attended several conferences of the committee with Dr. Williams. Since the laboratory routine in the small hospitals is extremely limited, Dr. Williams thought that the State might undertake to offer a course in laboratory technique which would be designed especially for training personnel for small hospitals. These courses would be much shorter than the year of training required by the Registry, and the curriculum would be much more limited in scope. We disagreed with this idea, for we felt that the small laboratories should be staffed by the most competent medical technologists who would be capable of working in isolated situations without the supervision of a pathologist and contact with other medical technologists. However, we could offer no other plausible solution to the problem. We reasoned that in order to plan a course it would be necessary to know exactly which procedures should be taught. Dr. Williams suggested that the committee survey representative small hospitals. He selected nineteen representative hospitals of 75 beds or less, and we helped compile the survey questionnaires. Visiting of hospitals and planning of itineraries for individuals was left to the committee. Letters of introduction from Dr. Williams were sent to each of the hospital administrators. This proved to be a great help in establishing friendly relations with the administrators and the laboratory personnel. It was made clear that it was merely a survey rather than an investigation of any kind. Armed with road maps, questionnaires, our most pleasant manners, and words of encouragement from Dr. Williams we embarked on our survey early in 1952. For most of the committee this glimpse into laboratories in small hospitals proved a real eye-opener, for the experience of committee members had been limited almost entirely to large hospital laboratories.

We submitted our reports which included a tabulation of laboratory work done in a typical month, information as to laboratory personnel, comments on the laboratory by the administrator, and the general impression of laboratory service by the technologist making the survey. Dr. Williams had the data compiled by a statistician and asked for a meeting of the committee to make our recommendations. Our most important recommendation was that the Division of Hospital Services employ a medical technologist as a laboratory consultant available to the laboratories in Georgia.

Upon our recommendation a qualified medical technologist was obtained to serve as Laboratory Consultant, full time. In the following article the Laboratory Consultant will discuss the work done in the past year.

LABORATORY CONSULTATION IN GEORGIA*

By (Mrs.) BETTY DEVON, B.S., M.T. (ASCP)
1017 Williams Mill Road, N. E., Atlanta, Georgia

In January, 1953, I accepted a position in Atlanta with the Division of Hospital Services, Georgia Department of Public Health, as Medical Laboratory Consultant. In my years of experience as a Medical Technologist, I had never heard of a Laboratory Consultant, thus I found myself with a job, wondering what to do about it.

The position of Laboratory Consultant was set up by the Division of Hospital Services to fill a definite need in the Hospital Construction Program under the Hill-Burton Act. The Division of Hospital Services has the responsibility for the allotment of Federal and State funds in Georgia, for hospital construction, and determines by a system of priorities the localities and institutions where such funds are to be allocated. Plans and specifications for the construction of new hospitals, as well as for enlargements and additions are reviewed to see that the requirements of the Federal Act administered by United States Public Health Service are met.

Many of the areas where these smaller hospitals are constructed have never before had a hospital facility in their community. There were few trained personnel available to select the necessary equipment to open the new hospital, and to successfully operate a new facility.

Experienced hospital personnel employed by the Division of Hospital Services to serve as consultants, include an accountant, dietitian, hospital administrator, medical technologist, nurse, and others. These consultants are available to all communities in Georgia, to assist in planning new hospital facilities or remodeling and improving existing facilities. Consultants assist in studying problems and make recommendations for setting up new procedures, or organizing various departments.

As Laboratory Consultant, several months were spent in visiting hospitals in Georgia, and trying to determine the areas in which a laboratory consultant was needed and could be most helpful. An urgent problem I found was for more and better trained laboratory personnel. As is well known there are not enough Registered Medical Technologists available, to place them in all hospitals. In many communities a local person has been trained by the physicians to do what the physicians consider to be essential laboratory work to meet the needs. There are a few hospitals fortunate enough to have well trained medical technologists; but even those technologists who are working alone in smaller hospitals have less opportunity to learn

*Read before ASMT convention, June, 1954, Miami Beach, Florida.

new techniques and keep abreast of progress in clinical laboratory methods.

In Georgia about 75% of the 250 hospitals have less than 50 beds. So, as you can see, trained personnel for the smaller hospitals is one of our main problems. Most of these hospitals employ one technologist who does both laboratory and x-ray work, seven days a week, on call 24 hours a day. The salaries offered in the smaller towns, generally are not sufficiently high to attract the better trained laboratory personnel, even if such technologists were available.

There are in Georgia about 22 pathologists of whom about 18 are directly affiliated with hospital clinical laboratories. Specialists locate in the larger cities, so obviously most of the work of Medical Technologists in the smaller hospitals is done without the direct supervision of a pathologist.

In August, 1953, Dr. R. C. Williams, director of the Division of Hospital Services called together a committee to discuss a program of Laboratory Consultation in Georgia, and especially to make recommendations as to the areas to be emphasized in this work. There were two basic ideas presented by the committee: First, a consultant should be available to all hospital technologists on an individual basis. Second, a consultant could plan and organize refresher courses or seminars for technologists, so as to make these services available to a larger number of technologists.

This planning conference was attended by representatives of the Medical Association of Georgia, Georgia Pathologists Association, Georgia Society of Medical Technologists, Georgia Hospital Association and the Division of Hospital Services, Georgia Department of Public Health. At this conference there was considerable discussion of the reasons for having a Medical Technologist as Laboratory Consultant. In Georgia, as in other states, I'm sure, there is a natural fear of anything in medicine that suggests "control", and to some of this committee, The State Health Department represented the threat of control. It was explained to this committee that the position of laboratory consultant was a service available without cost to hospitals on request, and that this service does not represent any type of supervision or direction.

The committee decided that emphasis should be placed upon reaching groups of technologists through refresher courses. Services of the Laboratory Consultant would also be available to an individual laboratory upon request. The committee also felt that the courses should be taught by Medical Technologists rather than Pathologists. Since emphasis was to be placed on improving technique, it was felt that Medical Technologists would be more intimately acquainted with the problems of technologists. The question arose as to the possible violation of the code of ethics of the Registry by those Registered Medical Technologists who would be teaching these courses. Not only would they be teaching without the direct supervision of a

pathologist, but also they would be accepting work outside their employers' practice since they would be paid an honorarium from the Georgia Department of Public Health for the time spent on this work. The pathologists present approved the plan for medical technologists to teach these courses if they were presented as refresher courses, open only to those laboratory personnel who had already had basic training in laboratory technique. These courses were not designed to prepare an individual for a career in laboratory work, but to review methods and discuss new techniques in the field of clinical laboratory procedures.

Upon the request of the laboratory consultant, the president of the Georgia Society of Medical Technologists appointed a special committee to work on these Regional Refresher courses. The committee was aware of the responsibility placed upon them and were determined to meet it. This committee of Medical Technologists decided that the first of this series of four refresher courses would be presented in Athens, Georgia, for the Northeast Hospital Council Area in October, 1953. It was felt that technologists in the smaller hospitals would not be able to get away for more than one day each week, and that the teaching material could not be adequately covered in less than five sessions. Thus five Fridays in October, 1953, were selected, and space in the Athens-Clark County Health Department was made available for these refresher courses.

The technologists on the committee agreed to assist in the teaching of the courses. It was decided that the usual routine procedures done in any clinical laboratory would be discussed and the program was planned to include one day of instruction on each of the following subjects: blood bank, hematology, biochemistry, urinalysis, bacteriology and parasitology.

Blood bank discussion included such matters as donor selections, ABO typing, Rh typing, Coombs tests, processing of blood, and other allied techniques.

Hematology was a review of techniques for white blood count, red blood count, hemaglobin, reticulocyte count, platelet count, and prothrombin time determination. The new terminology and classification of cells was discussed. Photomicrographs of blood cells were shown for group study of the morphology of blood cells.

Due to the fact that the State Health Department through its Central Laboratory in Atlanta and four branch laboratories in other sections of the State furnishes service for a wide variety of bacteriologic and serologic procedures, these subjects were not discussed at length. However, techniques for Gram stain, Zeihl-Neilson acid fast stain, and agglutination tests were presented, along with techniques for examining fecal material for ova and parasites.

In biochemistry, the methods of checking photoelectric colorimeters and spectrophotometers, and to a limited extent the physics of these instruments, was discussed. No attempt was made to stand-

ardize methods. The use of standard solutions available through the College of American Pathologists was explained along with directions for the use of these C. A. P. standards both for calibration of instruments and as daily standards. Techniques for blood sugar and non protein nitrogen were presented, as we find that these are two of the most commonly done biochemical procedures.

The routine urinalysis was discussed as were many of the less common procedures. We felt as a committee that this is one of the most common laboratory tests, and the one most likely to be neglected or given a "sink test" treatment. The technologist who presented this material found a unique method of making permanent preparations of urinary casts and other sediment in a gel solution, which were excellent for teaching purposes. Though these slides were made over six months ago they still show excellent detail. This method would be most helpful in any hospital where technologists are being trained, as rare casts or other urinary sediment may be preserved for teaching material.

This first series of refresher courses was our trial in more ways than one. The technologists who were teaching these courses were holding down regular jobs, and all of them spent many hours in preparation for teaching. As instructors they may have been overly zealous and super sensitive, but this first group for instruction was most difficult to approach, and seemed unresponsive to us. The "students" were requested to make comments and suggestions as these courses were being presented for their use, and they were our best critics. Many helpful suggestions did come from this original course. We were able to see many of our mistakes and plan the other three courses to the greater advantage of those on the receiving end.

In March, 1954, a Regional Refresher Course was presented in Albany, Georgia; at Swainsboro in April; and at Rome in May. These three series were presented on Wednesdays rather than Friday. The first group in Athens felt that the hospital work load was easier on Wednesdays.

The program was changed for these series to include fewer subjects and to give each subject more adequate coverage. Bacteriology and parasitology were dropped, as we felt that these subjects could not be adequately covered in the time allotted. The program then included one day each for blood bank, hematology, biochemistry and urinalysis. The fifth day in each series was left unscheduled in order that each group could select procedures in which they were particularly interested. These have varied and include such as prothrombin time, spinal fluids, blood alcohol, and special study of cells found in blood dyscrasias.

Our attendance at these courses has averaged 12 individuals per day. A total of 96 technologists have registered for these courses from about 50 hospitals, clinics and physicians' offices. We are told

that anything above 10% return of interest from people in a widely scattered area indicates excellent results. Based upon these figures we have had about a 20% return, whtich was quite pleasing.

Comments from those attending have been many and varied. Several students have felt that the material presented was too technical, especially in biochemistry, where calibration curves were discussed. Others have felt that more technical material should have been presented in bacteriology and serology. It has been somewhat difficult to hold the interest of these groups who meet only one day each week for five weeks. On this basis there has been a constantly changing group attending each session. We have encouraged group participation and invited discussion from those attending. This type of teaching is most difficult with almost a new group each day.

From this consultant's point of view, these refresher courses have at least stimulated interest among the technologists who are employed in smaller hospitals in geographic areas where they are rather isolated from the stimulus of working with other people who have similar professional activities. Many of those attending tell us that now they do not feel quite so alone in their laboratories, and have shown special interest in the continuation of these refresher courses.

Those attending this refresher training are constantly reminded that the services of the consultant are available to them on any problem they may have. This consultant is not always prepared to answer all the questions asked, or to offer the type of assistance needed. However, the Georgia Society of Medical Technologists have offered their services, and in fact, the job of laboratory consultation has been their job, rather than mine. They have given freely of their time, efforts and serious thought toward making this program a successful one. As a laboratory consultant and a member of the Georgia Society of Medical Technologists, we may look forward to better patient care through improved laboratory service in Georgia.

THE USE OF A QUANTITATIVE COOMBS TEST IN AN OBSTETRICAL SERVICE

MRS. VIRGINIA S. KYLE, M.T. (ASCP)
Herman Kiefer Hospital, Detroit, Michigan

Early in May of 1953 we started quantitative indirect Coombs tests on women selected by our obstetrical service and attending the clinic of that service. All of the cases tested were prior to delivery. Since the clinic opened eight years ago, we have been routinely testing all the clinic patients for blood grouping and Rh typing. After it was found that a woman was Rh negative, blood was drawn for a quantitative Coombs test on her next visit to the clinic. A few cases also had a saline and albumin titer run. However, the majority had only a quantitative indirect Coombs test run because of the small number of personnel in the blood bank. The results of the test went both to the clinic and also to the maternity ward so that the doctor would have adequate reports at the time of delivery.

Our method of testing was to set up serial two-fold dilutions of normal saline and the patient's sera in eleven test tubes starting with a 1:1, 1:2, 1:4, etc. dilution. We saved the dilution from the last tube in case the titer was higher. Starting with the 1:2 dilution we mixed the saline and sera well and transferred a two fold dilution to the next tube and so on through the eleventh tube. An amount of fresh group O positive cells (two per cent in normal saline) was added to each tube equal to the amount of normal saline used for each tube. It is best to use several group O positive bloods. The tubes with saline, sera, and O positive mixture were incubated for one hour at 37 C. in a water bath. After incubation, the tubes were examined for agglutination. The mixture was washed three times with normal saline. Two drops of Coombs sera were added to each tube and the directions of the manufacturer were followed for time and temperature of incubation. We used commercial sera for all of our tests.* Before reading, the tubes were centrifuged at 500 to 1000 rpm. for one to two minutes and read macroscopically using reflected light. We found that it was best to gently rotate the tube between our fingers to detect agglutination. The test was reported positive to the highest tube containing agglutination.

Our results and conclusions are from the laboratory findings only. Our study consists of the results of quantitative indirect Coombs tests for nine months. Of 125 tests, 73 were negative, 16 had a titer of 1:1, 4 of 1:2, 3 of 1:4, 6 of 1:8, 4 of 1:16, 5 of 1:32, 3 of 1:64, 2 of 1:512, 1 of 1:1424, 8 were beyond 1:1424. Several patients had from two to four tests during their pregnancy. One case (F. R.) showed a rise from 1:16 on 5-25-53 to 1:512 on

* Ortho Pharmaceutical Corporation, Raritan, New Jersey.

8-13-53. In the few cases where saline and albumin titers were also done, we found, in general, higher titers in the quantitative Coombs test. Undoubtedly a battery of tests on the Rh negative pregnant woman including saline and albumin titers, enzyme treated cells, conglutinin, and the indirect Coombs test is ideal. However, where there is a shortage of personnel, we believe the Coombs test is the test of choice since it alone detects all sensitivity on the red cell of the univalent type.

For a better understanding of the Coombs test we would like to review some of the principles of the test. The general use of the Coombs test started in 1945 for the purpose of detecting blocking antibodies. The sera is prepared by immunization of rabbits with human globulin and bleeding when there is sufficient titer. According to Coombs and Mourant the essential component of the anti-globulin or human sera is probably an anti-gamma globulin. When a blocking antibody is present, it is adsorbed on the surface of the red cell. The Coombs sera measures the specificity of adsorption. Rh positive cells are used for antigen. Thus the test demonstrated serologically the presence of sensitivity when blocking bodies obscure it. Sensitization occurs in the Rh negative mother when the fetus is Rh positive which is inherited from a Rh positive father. The mother may have been sensitized by previous Rh positive offspring or by transfusions or injections intramuscularly of Rh positive blood. In case of pregnancy the antigens in the Rh positive fetus cross the placental barrier and stimulate the production of antibodies by the mother which in turn cross the placenta from the mother. The reason the blocking antibodies cross the placenta barrier with more ease than the ABO antibodies is that these are univalent and simpler chemically than the polyvalent antibodies of the ABO groups which have larger molecular structures. There are wide differences of sensitization in Rh negative women but once sensitization is established it is permanent although there is a difference in degree. Positive Coombs tests have been found in fetuses ten to sixteen weeks after gestation.

Let us now limit our discussion of the Coombs test as an aid in the detection of possible hemolytic disease of the newborn by testing the pregnant Rh negative mother. The importance of studying and testing for univalent antibodies is that univalent antibodies are the primary cause of hemolytic disease of the newborn. In general all patients should be studied from the clinical and laboratory angles because of individual variations between laboratory findings and evidence and degree of hemolytic disease of the newborn. Even if titers are present it is impossible with present tests to detect the amount of antibody that crosses the placental barrier and how long the child has been

exposed to the antibody. Pickles states that the Coombs test gives the most reliable results and correlation for the detection of blocking antibodies.[1] Usually the disease is milder if saline agglutinins are predominately present and more severe if the titer is of the blocking variety. Also in general if a low titer is present for a short time before birth the disease is mild while high titers during the second trimester are more likely to result in death.

Summary

The use of the indirect quantitative Coombs test is described with results of 125 cases. A brief review of the principles of the Coombs test are given with a discusion of the use of titers in pregnant women for an aid in detection of hemolytic disease of the newborn.

Table I
Results of Indirect Quantitative Coombs Tests

Titers	
Neg.	73
1:1	16
1:2	4
1:4	3
1:8	6
1:16	4
1:32	5
1:64	3
1:512	2
1:1424	1
Above 1:1424	8

REFERENCES

1. Pickles, M. M.: Haemolytic Disease of the Newborn, Charles C. Thomas Company, Springfield, p. 47-49, 1949.
2. Mollison, P. L.: Blood Transfusion in Clinical Medicine, Charles C. Thomas Company, Springfield, p. 225, 226, 365, 370, 382, 1951.
3. Coombs, R. R. A.: In-vivo Isosensitization of Red Cells in Babies with Hemolytic Disease, Lancet, **250:** p. 266 (Feb. 23) 1946.
4. Hill, J. M. and Dameshek, W.: The Rh Factor in the Clinic and Laboratory, Grune and Stratton, New York, p. 82, 1948.
5. DeGowin, E. L., Hardin, R. C., Alserver, J. B.: Blood Transfusion, W. B. Saunders, Philadelphia, p. 103, 176, 1949.
6. Wiener, A. S. and Brancato, G. J.: Problems in the Management of Erythroblastosis Fetalis, with Five Examples Exhibiting Unusual Serological Findings, Journal of Laboratory and Clinical Medicine, **40:** p. 27 (July) 1952.
7. Wiener, A. S.: Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis) American Journal of Disease of Children, **71:** p. 17, 19, 40 (Jan.) 1946.

MALARIA*

By J. WALTER BECK, Ph.D.
University of Miami School of Medicine, Coral Gables, Florida

From the standpoint of medicine malaria still ranks as one of the most important of all diseases, in spite of the fact that we understand fully the nature of its cause and have made excellent progress in its control. Chandler, in his "Introduction to Parasitology," states that malaria has been estimated to be the direct or indirect cause of over one-half the entire mortality of the human race.

In distribution it is prevalent in nearly all tropical and substropical parts of the world, extending roughly between 45° north and 40° south latitude.

Early theories regarding the cause of malaria were numerous. The Latin derivation of the word malaria "mal-aria" indicates that the early observers associated this disease with the bad air coming from the swampy areas close to habitation. Little did they know that the air was bad because of the Anopheline mosquitoes which it bore. Other theories incriminated water, and it has been suggested that the early Roman aqueducts were built on account of the prevalence of "fever" in Rome. The discovery of the causative agent of malaria opened the field for investigation into the method of transmission, and it is to Sir Patrick Manson that much credit is due for, although he himself did not discover the method of transmission, since he worked with filarial worms he did give to medical science the discovery which was the foundation for the future work of Sir Ronald Ross. So revolutionary and so unimaginable was the mosquito theory of worm transmission of Manson that even the intelligent medical practitioners of the day would not believe him. The U. S. Navy Medical Inspector of the day, according to records, noted for posterity how the Navy surgeons of different countries, when coming together for a meeting, used to chaff and ridicule Dr. Manson about his mosquito filaria ideas. They called him "crazy Pat Manson" and "mosquito Manson." His work was brilliant and revolutionary and he deserves even greater honor than is accorded him considering the apathy and discouragement of his colleagues who even questioned the sanity of his mind.

The malaria parasite was first described in 1880 by Laveran, a French Army Surgeon studying malaria in Algeria. The life cycle in the red blood cells was soon worked out, but the mystery of how man contracted the disease still remained unanswered. Sir Patrick Manson, fresh from his success with the mosquito transmission of filarial worms, insisted upon the necessity for experimentation with mosquitoes and formulated the hypothesis that they might be the necessary secondary hosts. It was largely due to Manson's suggestion

*Read before ASMT convention, June, 1954, Miami Beach, Florida.

that Dr. Ronald Ross, then a surgeon in the Indian Medical Service, began his studies.

With nothing but a theory, no knowledge whatever of what the organism might look like if it were present in the mosquito or what part of the body it might be in, or even what species of mosquito might be involved, Ross spent two and one-half years of strenuous work before he finally solved the problem and found the parasites among the cells of the stomach wall of what he termed "the dapple-winged mosquito" we know as *Anopheles quadrimaculatus.* Just as Mendel was fortunate in choosing the right plant to work on in establishing his laws of inheritance so Ross was fortunate in choosing his mosquito to prove a theory. Had he chosen any one of many other species he would have met with failure and the method of transmission would have remained unknown until a future date. Ross's work was one of the great discoveries in biological science applied to medicine. It was the forerunner of the great antimosquito control programs that were to follow and, because of Ross's discovery, malaria is now almost extinct in the U. S.

For a long time it was though that only two phases were involved in the life cycle of the malarial parasite, one in the mosquito and the other in the red blood cells of man. We now know that it is not quite that simple.

When Schaudinn first stated that he saw sporozoites from the mosquito enter the red blood cells of man the idea seemed quite plausible and was readily accepted though not verified at the time. Schaudinn himself could not duplicate his experiment and no worker since has observed this phenomenon. Not until quite recently has this phase of the cycle become known. With the knowledge gained from the study of malaria in birds and monkeys pieces of the puzzle are gradually being put in place and Shortt and his co-workers in 1948 finally succeeded in demonstrating parasites in the parenchymal cells of the liver of a human volunteer after inoculation with sporozoites and prior to the presence of parasites in the red blood cells.

During and since World War II tremendous work has been done in the realm of malarial therapy and new drugs periodically appear. In spite of our knowledge of control and treatment of malaria still ranks as one of the most important of all diseases.

Some of you may be of the opinion that since malaria is almost extinct in this country, there is little need to worry about its diagnosis in your laboratory. Such is far from the truth. In this age of air travel and the dissemination of military personnel to the far-flung corners of the earth, imported cases are quite common and sporadic outbreaks occur from time to time in the civilian population. The perfect drug is yet to be found and accurate diagnosis of species is important. As technologists you should be familiar with the thick smear method of diagnosis. When in doubt thin smears may be used to determine the species.

GERMICIDAL EFFECTIVENESS OF FORMALDEHYDE FIXATIVES AND PRESERVATIVES AGAINST BACILLUS ANTHRACIS IN ANIMAL TISSUE

By G. BRIGGS PHILLIPS, B.S., FRANK NOVAK, B.S., and EVERETT HANEL, Jr. B.S.
Camp Detrick, Frederick, Maryland

Introduction

It is generally assumed that infected animal tissues are sterilized by formaldehyde fixation. Meade and Steenken (1949) were unable to isolate viable tubercle bacilli from lung tissues of tuberculosis cadavers embalmed with formaldehyde fluids 3 to 48 months previously. These authors cited previous tests in which tubercle bacilli remained viable in an unembalmed, refrigerated human body for at least three months. In contrast, Weed and Baggenstoss (1952) demonstrated viable tubercle bacilli as well as a variety of other organisms in various tissues and body fluids of cadavers which had been embalmed from 24 to 48 hours and in one case 60 hours. From the tissue and body fluids of 25 specimens these authors isolated and cultured a variety of common organisms. In addition *Mycobacterium tuberculosis* was cultured from 22 specimens and *Histoplasma capsulatum* from one specimen. It was suggested that such embalmed materials might be the source of tuberculosis or other infections among pathologists or other persons handling cadavers.

After considering the above reports here it seemed desirable to investigate the hazards involved in the handling of animal tissues and organs removed from infected animals at autopsy for histological examination. The present investigation is concerned with the bactericidal action of formaldehyde on infected animal tissues since formalin constitutes the active germicidal component of many fixative and preservative solutions used before histological examinations. The action of formaldehyde solutions was determined on *Bacillus anthracis* organisms from experimentally infected mouse tissue moved at necropsy for subsequent histological examination.

Experimental Methods

White mice weighing between 16 and 18 grams were inoculated subcutaneously with approximately 1×10^{6} *B. anthracis cells.* After death, the liver and spleen from each mouse were removed and a portion cultured for *B. anthracis.* The remainder of these organs were placed into a single bottle containing an aqueous solution of formaldehyde which was tested in concentrations of 0.1, 1 and 5 per cent. The tissues were immersed in the solution for 24 hours at 5° or 20° C and subsequently cultured.

Before culturing the treated tissues, the formaldehyde was inactivated with a solution containing 11 grams of sodium sulfite and 6 grams of ammonium chloride per 100 ml of water. Control studies were performed on the possible bactericidal action of the neutralizing solution and the extent of its formaldehyde neutralization capability.

The liver and spleen from normal mice were immersed in a 5 per cent formaldehyde solution for 24 hours and then ground in a mortar containing 2 ml of the sodium sulfite-ammonium chloride solution. One ml of the resultant suspension was mixed in a test tube with 1 ml of *B. anthracis* cells, suspended and then 0.1 ml of the mixture was plated on the surface of a medium composed of Difco blood agar base with 5 per cent sheeps blood. The number of organisms exposed was adjusted so as to obtain a countable number of colonies on the subculture plates. Counts after 48 hours incubation at 37° C were compared with counts obtained when saline was substituted for formaldehyde in the procedure cited above. Five tests carried out in this manner showed that 78 per cent of the organisms exposed to the mixture of formaldehyde and its inactivator were recovered, which is an adequate number for these tests.

In attempting to recover *B. anthracis* from the treated tissues the liver and spleen of each infected mouse were removed from the formaldehyde solution and placed in a mortar containing 2 ml of the NA_2SO_3-NH_4CI solution. The tissues were ground thoroughly and the resulting emulsion cultured on Difco blood agar base with 5 per cent sheeps blood. Culture plates were examined for typical *B. anthracis* colonies after 48 hours incubation at 37° C. In some tests Difco tryptose broth also was used as a subculture medium.

Since the presence of spores of *B. anthracis* might reduce the effectiveness of formaldehyde, an attempt was made to ascertain the possible presence of sporulation of the test organisms in tissues after death of the test animal. Sections of liver and spleen were removed from mice dead of anthrax, placed in Petri dishes and held at 20° or 5° C for up to 48 hours. Tissues were examined for spores by emulsifying a small portion in saline and preparing methylene blue stained smears. No spores could be detected in the tissues by this method.

Results

Table 1 shows the results obtained with three concentrations of formaldehyde. Tissues exposed for 24 hours to 5 and 1 per cent formaldehyde at 20° C and tissues exposed to 1 per cent formaldehyde at 5° C for 24 hours were sterile. Viable spores were recovered from 4 out of 10 specimens exposed to 0.1 per cent formaldehyde for 24 hours at 20° C.

Table 1. Recover of *B. anthracis* from infected spleens and livers after exposure to formaldehyde solutions

No. of Tests	Concentration of HCHO	Temperature	Percent Tissues Positive
10	5%	20 C	0
10	1%	20 C	0
10	1%	5 C	0
10	0.1%	20 C	40

Exposure time for these tests was 24 hours.

Summary

Possible hazards involved in the handling of infected animal tissues treated with formaldehyde have been investigated. The spleen and liver of mice dead of anthrax were immersed in various aqueous solutions of formaldehyde for 24 hours. It was found that *B. anthracis* could not be cultured from tissues when a concentration of formaldehyde as low as 1 per cent was used. These results indicate that common fixative and preservative solutions containing formaldehyde and used in a standard manner will kill all *B. anthracis* organisms in infected mouse livers and spleens.

REFERENCES

1. Meade, G. M., and Steenken, W., Jr.—Viability of tubercule bacilli in embalmed human lung tissues. Am. Rev. Tuberc., 59: 429-437, 1949.
2. Weed, L. A., and Baggenstoss, A. H.—The isolation of pathogens from embalmed tissues. Proc. Staff Meeting of Mayo Clinic, 27:124-130, 1952.

A LABORATORY MOTION INVENTORY

By BEVERLY BUCKNER, B.S., M.T. (ASCP)
Caylor-Nickel Clinic, Bluffton, Indiana

As a laboratory increases in size and scope and adds or eliminates tests, it is easy for the workers to become so involved in the tangle of tests that they do not have or take the time to evaluate their efficiency. Frequently, it takes a visitor or someone detached from the work at hand to see where time and motion are lost due to inconvenient arrangement of work space, scattered chemicals, disorganized grouping of glassware, and poorly placed electric and gas outlets. It is well worth the time it takes to uncover and eliminate bottlenecks, wasted motions and time consuming tasks that are unproductive. Here are some suggestions for taking a motion inventory:

In order to discover the weaknesses of a given laboratory or a given department of a laboratory, first determine which tests are done most frequently, and second prepare a diagram or "flow sheet" of the working area where each test is performed. In this case, a "flow sheet" is a floor plan of the working area on which all steps necessary to perform any one given test are marked. For example, in the following "flow sheet" the steps necessary to perform a blood sugar are diagramed starting at the point labeled "blood drop," the place where the sample is deposited after drawing. From the "blood drop" the steps continue to the workbench where the filtrate is prepared. All motions necessary

to prepare the filtrate are marked if they represent steps on the part of the worker. Next are charted the steps necessary to get a sugar tube, to add the filtrate to the tube, and to place the tube in the boiling water. Also included are the steps necessary to add the additional reagents, reading the results in a colorimeter, recording the results and finally clearing the area of dirty glassware. After diagraming the necessary steps in several tests, a flowing or general movement of the tests toward a given spot can be seen. With this general direction in mind, the workers can eliminate those spots which show evident backtracking or are bottlenecks when two or more people are working in the same area. Also to be avoided is the tendency for workers to rotate around each other. This may be done by starting several tests on the same sample at the same time, and then moving each test on to the succeeding steps as time and procedure demand. For example, in those laboratories which use the Folin-Wu filtrates for several tests and which perform blood urea nitrogen determinations frequently one person can prepare blood sugar and uric acid filtrates and start the blood urea nitrogen test all at the same time.

With convenient glassware, pipets and reagents, many serum tests can be set up simultaneously, and partially eliminate the need for more than one person to handle a specimen.

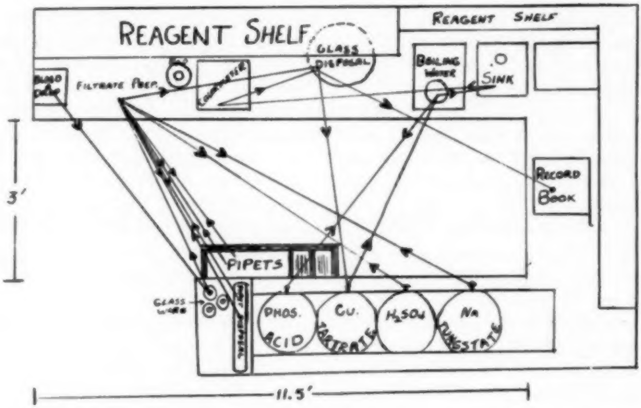
Of major importance also is the location of receptacles for dirty glassware and pipets. These receptacles should be placed as conveniently as possible. With the new plastic jars being resilient to bumps and jabs and less likely to break pipet tips when they are dropped in, there should be many more convenient places available.

Realizing that the type of laboratory, whether it is clinic, hospital or private, and the type of work done will present many varied problems, the following diagrams are merely examples of how a "flow sheet" will help point out rough spots in a chemical department.

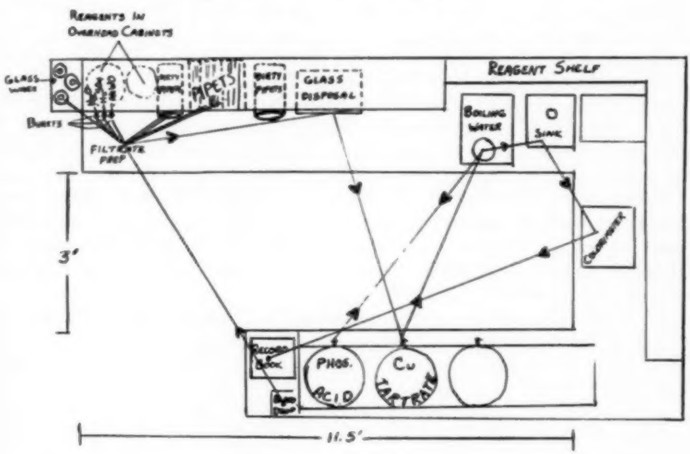
It should be pointed out that much confusion is eliminated by bringing the record book out of a pocket. The new location of the book gives everyone easy access and eliminates heavy traffic through the working area.

Another feature of this reorganized department is the overhead feeding system which provides, without any decrease in accuracy, measured amounts of distilled water and other chemicals used in quantity. By means of large burets attached to a supply in the cabinets over the working counter, distilled water, sulfuric acid and sodium tungstate solutions are introduced into the work area at the time of their use. The worker making filtrates can reach all the necessary materials and supplies with-

Original "flow sheet" for a Blood Sugar:



Reorganized "flow sheet" for a Blood Sugar:



out moving from one spot. This noticeably increases the speed of the worker and reduces the number of trips across the three foot aisle between the work counter and the reagent table from

sixteen to six trips. The reduced number of trips in turn reduces the chance of two workers running into each other or being in each other's way.

While a "flow sheet" will not solve all the bottlenecks and conserve all wasted motion, it will help point the way to a more efficient laboratory and consequently, more effective use of trained personnel.

ACKNOWLEDGMENT

This is to acknowledge the kind assistance of Dr. John A. Shively, Pathologist of the Caylor-Nickel Clinic, in the preparation of the paper.

DETERMINATION OF BLOOD CHOLESTEROL*

EVANGELINE PAPAGEORGE, Ph.D.
Department of Biochemistry, Emory University, Georgia

This discussion is primarily a review of problems involved in application of the Liebermann-Burchard reaction to the determination of serum cholesterol. The reaction was first observed by Liebermann in 1885 (1) and applied by Burchard to chloroform solutions of cholesterol in 1890 (2). It involves the formation of a green color upon addition of acetic anhydride and concentrated sulfuric acid to solutions of cholesterol under anhydrous conditions. Reference will be made to a number of procedures for free and esterified cholesterol of serum, as well as for total cholesterol, a few of which employ another type of color reaction. Details will be given for a relatively simple technique for total cholesterol which was devised about six years ago by the author for use in the Emory University Hospital Laboratory and has been adopted in a number of clinical laboratories in metropolitan Atlanta. This procedure involves no original device but incorporates features from several other methods and is presented only as one of the less complicated techniques which in the hands of the average technician can give reasonably accurate and quite reproducible results.

In clinical analysis, serum or plasma cholesterol is of greater interest than values for whole blood since it is in the former that pathological alterations are best seen. The red cells contain only free cholesterol and this in quite constant concentration. The plasma, however, normally has 68 to 76% of its cholesterol in esterified form. In liver and biliary disease, this ratio of free cholesterol to ester may be increased, whereas in other conditions associated with abnormal serum cholesterol values, this ratio is usually undisturbed. Thus the medical technologist is most frequently concerned with the determination of total serum cholesterol, but on occasion is called upon to analyze also for free or

* Read before A.S.M.T. Convention, Miami Beach, Fla., June 1954.

esterified sterol as well. In the determination of free cholesterol, use is made of its property to form an insoluble compound with digitonin, a highly hydroxylic glycoside. For the formation of the digitonide, the sterol must be free, and the free OH group on the 3-position of the A ring must be in the same plane as the methyl group attached to carbon-10. Cholesterol is the only sterol with this configuration which is present in appreciable amounts in serum.

Most reliable procedures require preliminary extraction of the serum with some fat solvent or solvent mixture which simultaneously precipitates the serum proteins. Bloor's 3:1 alcohol-ether mixture (3) has been popular, and has the advantage of providing an extract suitable for differential lipid analysis. However, if digitonin is to be employed for precipitation of free cholesterol, it is recommended that alcohol-acetone be used for extraction (4,5) to remove phospholipids which may prevent complete precipitation of the digitonide. Recently, at least two methods for total cholesterol have been proposed in which no preliminary extraction is required. One of these (6) employs *p*-toluenesulfonic acid for correction of interfering substances and applies the Liebermann-Burchard reaction directly to 0.1 ml. of serum mixed with 0.1 ml. of glacial acetic acid. Another (7) uses a new color reagent, namely 10% ferric chloride in glacial acetic acid, diluted 100-fold with concentrated sulfuric acid. This reagent is added directly to 0.1 ml. of serum previously mixed with 3.0 ml. of glacial acetic acid. In very limited experience with these two methods, we have been unable to obtain accurate results (by comparison with other methods and in recovery experiments), probably because the techniques as published do not provide sufficient detail of procedure. Should these methods prove capable of giving reasonably accurate values, they offer great advantages in speed and simplicity of procedure and in the small amounts of serum required for the determination of total cholesterol. Where free or esterified cholesterol is to be also determined, however, preliminary extraction of serum is called for in all methods known to the author.

After a suitable extract containing the cholesterol (along with other serum lipids) has been prepared, a variety of procedures have been proposed for isolation of cholesterol before carrying out the color reaction. The Liebermann-Burchard reaction is not specific for cholesterol and, furthermore, takes place more rapidly with cholesteryl esters than with the free sterol under the same conditions. Temperature, light, and the solvent employed in the last step all play a role in the rate at which maximum color is achieved and in the stability of the color obtained. The various techniques thus differ largely in the following features: whether or not saponification is employed to free the cholesterol esterified

with fatty acids; the use of digitonin and exact conditions of digitonide precipitation and purification; the nature of final solvent in which the color reaction is carried out; details such as temperature, exposure to light, time of reading, etc.

For very precise analysis, and particularly where the ratio of free to total cholesterol is desired, the method par excellence remains that of Schoenheimer and Sperry (4), as modified by Sperry and Webb (8). This is the method presented for total and free cholesterol in the manual of standard methods published by the American Association of Clinical Chemists (9). The section on cholesterol in this manual (pages 43-54) is recommended for a clear presentation of details of procedure and for an excellent discussion of the significance of serum cholesterol values as well. The method involves saponification of an aliquot of the acetone-alcohol extract of serum followed by acidification with 10% acetic acid and subsequent treatment with alcoholic digitonin for isolation of all of the cholesterol as its digitonide. For free cholesterol determination, the digitonide is precipitated directly from another aliquot of extract which has not been saponified. The digitonide is isolated by centrifugation, washed with suitable solvents, and evaporated to dryness. It is then dissolved in glacial acetic acid and submitted to the Liebermann-Burchard reaction at 25° in the dark and assayed photometrically 27 to 37 minutes after addition of the reagent. The difference between total and free cholesterol values is that of esterified cholesterol.

Among somewhat simpler versions for total cholesterol which likewise advocate preliminary saponification are those of Kelsey (5), Sperry and Brand (10) and Abell *et al.* (11). Kelsey includes a procedure for free cholesteral in his method. Kaye (12) and Sobel and Mayer (13) have proposed a much simpler modification for the determination of free cholesterol following isolation of its digitonide. Zak and his co-workers have very recently applied their color reaction to a rapid method for free cholesterol (14). An interesting departure from the Liebermann-Burchard reaction has been the application of the anthrone reaction to cholesterol digitonide, suggested for free and total cholesterol by Feichtmeir and Bergerman (15). The color developed in this reaction is stable and less subject to the influence of temperature. While the author has had no personal experience with this method, a colleague reports that the procedure as published needs some refinement but offers definite promise of advantages over methods based upon the Liebermann-Burchard reaction.

All of the procedures involving saponification are more or less time-consuming, and present a problem in a laboratory carrying a heavy load of routine analyses. Following saponification, the cholesterol must be separated either by isolation of the digi-

tonide after careful acidification (4,5,8,15), or by extraction with suitable solvents from the saponified mixture (10,11). Thus, many laboratories prefer techniques in which no saponification is required for routine determination of total cholesterol. In this category fall procedures such as those of Kaye (12) and Kingsley and Shaffert (16), as well as that proposed by the author below. While admittedly, results by a Liebermann-Burchard method without previous saponification give results that are generally 10 to 20 mg. % higher, this order of error is seldom significant in routine determinations for total cholesterol and this disadvantage is balanced by the saving in time and greater simplicity of procedure which makes for more accurate operation by the average technician.

The Kingsley and Shaffert method is one of the most attractive versions of the Liebermann-Burchard reaction. For total cholesterol, serum is extracted directly with chloroform. Treatment with magnesium sulfate accomplishes the dual purpose of assisting precipitation of proteins and removing water which interferes in the color reaction. Fuller's earth is also used before centrifugation and an aliquot of the supernatant is submitted directly to treatment with acetic anhydride-sulfuric acid reagent at room temperature and in the absence only of direct sunlight. Kingsley and Shaffert give a similarly simple technique for the determination of ester cholesterol, following quantative removal of the free sterol as its digitonide from the chloroform extract at the time of protein precipitation. One of the main objections to the Kingsley-Shaffert procedure is the several 5-minute shaking periods attending the extraction operation, unless a suitable shaking machine is available. While Kingsley and Shaffert do not prescribe routine saponification, they recommend the use of a correction factor to rule out the greater colorigenic value of esterified cholesterol. This factor, they suggest, should be determined in each laboratory by analyzing a series of sera both with and without hydrolysis. For many laboratories this step may be too inconvenient to make the method attractive.

The technique presented below involves application of the Liebermann-Burchard procedure to an alcohol-ether extract prepared according to Bloor (3) following evaporation of an aliquot much as in the Sackett method (17). However, glacial acetic acid is substituted for chloroform as the solvent for cholesterol before treatment with the Liebermann-Burchard reagents in order to permit use of the Schoenheimer-Sperry standard in glacial acetic acid (4). This was done because of the far greater stability of cholesterol in glacial acetic acid as compared to solutions in chloroform. The sacrifice entailed in less rapid development of color is negligible. In the color reaction, the mixed reagent recommended by Sperry and Brand (8,9,10) is used to

ensure uniform addition of sulfuric acid to all tubes. Recovery of cholesterol added during the extraction step has been tried with good results.

Reagents:

1. Alcohol-ether mixture: 3 parts of 95% alcohol to 1 part of anhydrous ether (high-grade purity, or re-distilled).
2. Glacial acetic acid (reagent grade).
3. Acetic anhydride (reagent grade). Purchase in small bottles and keep well-stoppered in cool place.
4. Concentrated sulfuric acid (C.P.)
5. Acetic anhydride-sulfuric acid mixture. 20 volumes of anhydride are mixed with one volume of concentrated sulfuric acid as follows. Place the 20 volumes of acetic anhydride in a previously chilled flask provided with a glass stopper and chill contents thoroughly in an ice bath. Keep the flask in the ice bath, and add 1 volume of concentrated H_2SO_4 *cautiously,* agitating the flask during addition of the acid. Insert stopper, shake vigorously and return to ice bath. Wait at least 9 minutes before using. The anhydride-acid mixture will keep 1 to 1½ hours in the ice bath, but should not be used if more than 90 minutes have elapsed since the mixing of the reagents.
6. Cholesterol standards.
   Stock: 100 mg. of pure anhydrous cholesterol made up to 100 ml. with glacial acetic acid. If necessary, warm to facilitate solution before making to volume. Store in glass-stoppered bottle. Keeps at least 1 month. 1 ml. = 1 mg. cholesterol.

Since cholesterol does not keep well under ordinary conditions, it should be recrystallized and dried thoroughly before used to prepare standard solutions. Cholesterol may be recrystallized from absolute ethanol, from methyl alcohol, or from ethylene dichloride. Example: dissolve 1-2 gm. of cholesterol in a minimum of hot absolute ethanol; cool and collect crystals which separate on standing on a small Buchner filter; wash several times with small quantities of cold, absolute ethanol, and dry by suction: Store in vacuum dessicator over phosphorus pentoxide or concentrated sulfuric acid. *Dilute working standard:* Dilute 1 volume of stock to 5 volumes with glacial acetic acid and place in tightly stoppered botle. Keeps at least 2 weeks. 1 ml. = 0.2 mg. cholesterol.

Procedure:

Since the alcohol-ether mixture is highly inflammable, all steps in which this mixture is involved must be performed in a room in which there are no flames, and a hot plate should be used for heating.

1. Place 1 ml. of serum in a 25-ml. volumetric flask fitted with a glass or cork stopper, add 15 ml. of the alcohol-ether mix-

ture with a graduated cylinder, and agitate vigorously for a few minutes to effect thorough mixing.

2. Place the *unstoppered* flask in a boiling water bath on a hot plate, and rotate the flask rapidly to prevent bumping. As soon as the mixture boils, remove from the water bath and cool to room temperature. Make to volume with alcohol-ether, stopper, and mix. Filter the extract through a Whatman No. 1 filter paper (fat-free) into a small Erlenmeyer flask, keeping mouth of funnel covered with a watch glass to avoid evaporation. Stopper the flask tightly (cork or glass stopper) and place in the refrigerator until the analysis can be continued.
3. Measure 5 ml. aliquots of the alcohol-ether extract into clean, dry colorimeter tubes containing a glass bead each and place in a deep hot water bath on a hot plate. Evaporate carefully just to dryness, watching closely to prevent bumping. Avoid charring.
4. Dissolve the dry residue by addition of exactly 2 ml. of glacial acetic acid. To facilitate solution, agitate the tubes in a hot water bath. (Tubes may be left in a hot though not boiling water bath for as long as 30 minutes; however this is seldom necesary.) Cool tubes to room temperature before addition of the color reagent. If the analysis must be interrupted at this stage, stopper tubes tightly with cork and keep at room temperature until analysis can be completed on the next day.
5. Prepare blank and standard tubes. For the blank, measure 2 ml. of glacial acetic acid into a clean, dry colorimeter tube, and for the standard use 2 ml. of the working cholesterol standard (1 ml. = 0.2 mg.) in a similar tube. Place all tubes (blank, standard and cooled unknown tubes, in a water bath at about 25° C. and allow to come to the temperature of the bath. When the tubes have all attained the temperature of the bath, carry out the Liebermann-Burchard reaction as described below. Treat one tube at a time and allow the proper interval of time between addition of the anhydride-acid reagent to the tubes, since the time between addition of the reagent and the colorimetric reading (to be made about 30 minutes later) must be the same for all tubes.
6. Remove each tube from the bath, wipe the outside dry, and add exactly twice its volume (4 ml.) of the cold anhydride-acid reagent from the burett with a glass stopcock *(do not pipette the reagent)*. Note time, mix thoroughly and return tube to the water bath (about 25°) in a dark cabinet or box. Allow to stand in the dark for 27-37 minutes (preferably 30 to 31 minutes after addition of reagent for each tube) before colorimetric assay.
7. Place red filter (660 millimicrons maximum) in photoelectric colorimeter, or set spectrophotometer dial to 660, (although, 625 millimicrons gives satisfactory readings). Adjust to 100%

transmission (zero density) with blank (this may be done before 27 minutes have elapsed since addition of the reagent). Read standard and unknowns, and calculate.

Calculation:

$\frac{\text{(Optical density)}_x}{\text{(Optical density)}_s} \times 0.4 \times 5 \times 100 =$ mg. cholesterol per 100 ml. serum.

REFERENCES

1. Lieberman, C., Ber., **18:** 1803-1809, 1855.
2. Burchard, H., Chem. Zentr., **61** (1): 25-27, 1890.
3. Bloor, W. R., J. Biol. Chem., **77:** 53-73, 1928.
4. Schoenheimer, R., and Sperry, W. M., J. Biol. Chem., **106:** 745-760, 1934.
5. Kelsey, F. E., J. Biol. Chem., **127:** 15-22, 1939.
6. Pearson, S., Stern, S., and McGavack, T. H., Anal. Chem., **25:** 813-814, 1953.
7. Zlatkis, A., Zak, B., and Boyle, A. J., J. Lab. and Clin. Med., **41:** 486-492, 1953.
8. Sperry, W. M., and Webb, M., J. Biol. Chem., **187:** 97-106, 1950.
9. American Association of Clinical Chemists, Standard Methods of Clinical Chemistry, Vol. I, Academic Press, New York, 1953, pp. 43-54.
10. Sperry, W. M., and Brand, Florence C., J. Biol. Chem., **150:** 315-324, 1943.
11. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., J. Biol. Chem., **195:** 357-366, 1952.
12. Kaye, I. A., J. Lab. and Clin. Med., **25: 996-1001,** 1940.
13. Sobel, A. E., and Mayer, A. M., J. Biol. Chem., **157:** 255-264, 1945.
14. Brown, H. H. Zlatkis, A., Zak, B., and Boyle, A. J., Anal. Chem., **26:** 397-399, 1954 (Feb.).
15. Feichtmeir, T. V., and Bergerman, J., Technical Bulletin of the Registry of Medical Technologists (ASCP), **23:** 101-104, 1953 (May).
16. Kingsley, G. R., and Schaffert, R. R., J. Biol. Chem. **180.** 315-328, 1949.
17. Sackett, G. E., J. Biol. Chem. **64.** 203-205, 1925.

SOME PRECAUTIONS RECOMMENDED IN EXFOLIATIVE CYTOLOGIC TECHNICS*

By CHARLOTTE M. STREET, B.S.

*Papanicolaou Cytology Laboratory***
Cornell University Medical College, New York, N. Y.

The success or failure of many a diagnostic test depends upon the observance of certain "do's" and "don't's" both in the manner of obtaining specimens and in the performance of laboratory procedures. The preparation of smears for cytologic examination for the diagnosis of cancer is no exception. On the contrary, while the technic of collecting and processing specimens is only a part of cytologic test, and the least difficult part at that, accurate microscopic interpretation is impossible unless the smears are properly prepared. Since most of the criteria of malignancy used in the evaluation of exfoliated cells are based on morphologic characteristics, the cells must be well preserved and well stained to show their structural details to best advantage.

In regard to the adequacy of cellular material, the full responsibility does not rest entirely with the technologist but often must be shared by the individuals who obtain the specimens, since proper methods of collection and immediate fixation are important. Regardless of how perfect the staining technic may be, poorly fixed cells will not take stains properly and cannot be accurately interpreted. The cooperation of the physician must therefore be obtained in procuring good specimens.

I. Collection of Specimens:

In collecting the various specimens, there are several precautions to be recommended which, if followed, should provide suitable material for study and therefore result in greater diagnostic accuracy.

A prerequisite for a suitable preparation from the female genital tract, for instance, is the avoidance of douching by the patient for several hours prior to the test so that the presence of ample quantities of exfoliated cells will be assured. No lubricant should be used as it distorts and partially obscures the cells in a stained smear. Although the need for a cytologic examination is not always anticipated, it is advisable to prepare smears before the pelvic examination whenever possible in order to avoid the presence of lubricant and any possibility of traumatic bleeding which would dilute the cellular content of the secretion. Smears should not be prepared less than two weeks after a cauterization or curettage as the tissues undergo changes following such pro-

* Read before the Empire State Association of Medical Technologists, New York, N. Y. April 24, 1954.

** The work of this laboratory is supported in part by a Control Project Grant (C8454) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

cedures, and the exfoliated cells are often altered and show atypical forms. Following uterine curettage, for instance, endometrial cells may show marked nuclear activity due to the regenerative process. In addition, they often contain cytoplasmic vacuoles and engulfed leukocytes, and form clusters resembling to some degree those commonly exfoliated from an adenocarcinoma of the endometrium. To the inexperienced, they may easily be misinterpreted as malignant. It is also inadvisable to make smears if the patient is bleeding profusely as the cellular concentration is greatly reduced and the epithelial cells which may be present will be partially obscured by blood.

A few suggestions might be given as to methods for obtaining the various secretions from the female genital tract. The pipette and bulb used for aspirating vaginal secretions, and the cannula and syringe used for endocervical or endometrial aspirations[1] must be dry. For endometrial aspirations, the cannula should also be sterile and the usual sterile precautions are to be observed. Slides should be cleaned and labelled in advance as to the type of smear. For vaginal smears it is best to aspirate the secretion from the posterior fornix where exfoliated cells are most likely to accumulate, but the pipette should be moved about so as to obtain an adequate sampling of the vaginal contents. Material can be obtained from the cervix in the region of the squamo-columnar junction by either swabbing or scraping,[2] but the latter should not be too vigorous as it may result in bleeding. In order to obtain pure endocervical or endometrial secretions it is recommended that a separate cannula and syringe be employed for each, and suction applied only while the cannula is within the cervical canal or the uterine cavity. Endometrial smears should, of course, be omitted if there is an infection or suspicion of pregnancy.

It is not always possible to obtain all four types of gynecological smears, i. e., vaginal, cervical, endocervical and endometrial, but the more areas that can be sampled cytologically, the greater will be the chance for diagnostic accuracy.

In the actual preparation of gynecological smears, the secretion should be spread uniformly and fairly thin, since thick smears cannot be well penetrated by the fixing, staining and clearing solutions. Moreover, in thick preparations the cells are apt to lie in dense clumps thereby causing difficulty in the study of individual cells. Swab and scraping smears sometimes show cellular distortion which may be caused by using too much pressure in spreading the material. All smears should be immersed immediately in a solution of equal parts of ether and 95% alcohol before any drying takes place. This is very important because cells which have been allowed to dry appear enlarged

or otherwise distorted and their nuclear structures are poorly defined since their affinity for stains has been reduced.

Smears from secretions of organs other than the female genital tract are usually prepared in the laboratory and it is the responsibility of the technologist to see that they are made properly. There are, however, a few suggestions which may be made as to the technics of obtaining and preserving specimens before they reach the laboratory. The most important thing is immediate fixation of the cells by mixing the specimen with alcohol as soon as it is obtained from the patient, and delivery to the laboratory without delay.

Specimens from the respiratory tract may include sputa, bronchial aspirates and bronchial washings. Sputum is collected in a wide mouthed bottle containing about 30 cc. of 70% ethyl alcohol. The patient must be impressed with the importance of producing a good, deep cough specimen, preferably upon arising. Salivary specimens are obviously of no value in a search for pulmonary carcinoma cells. Smears may be more easily prepared from fresh unfixed sputum but unless such material is processed quickly, growth of bacteria and fungi as well as degeneration of the cells will result. It is therefore preferable to have suptum collected directly in alcohol. 70% alcohol is used for this as 95% hardens the sputum and makes it difficult to spread. Bronchial aspirates or saline washings are mixed immediately with an equal amount of 95% alcohol. The collecting tube should be rinsed well with saline or alcohol and the rinsings added to the specimen, since the volume of such secretions is usually small, and care must be taken to obtain as much of the available material as possible.

From the genito-urinary tract the following specimens may be obtained: voided or catheterized bladder specimens, ureteral specimens, urine voided before and after prostatic massage and smears of prostatic secretion. Urine from females should always be catheterized to avoid contamination by cells from the genital tract. In males where prostatic cancer is suspected and the prostate is massaged to obtain specimens, the massage must be gentle in order to avoid the spread of carcinoma cells. In making prostatic smears, it is advisable to spread the secretion uniformly on a slide which has been coated with a thin film of Mayer's albumen to form an adhesive surface for the cells. The smear is then fixed in ether-alcohol without drying.

One regrettable error in diagnosis was made a few years ago, and although there is no guarantee against its repetition, we are now aware at least of the possibility of such an error. From a case in which there was a clinical suspicion of carcinoma of the kidney, one ureteral specimen was submitted for cytologic ex-

amination and was reported as positive for malignancy. Because of the clinical suspicion and the positive smear report, the kidney was removed but no tumor could be found. Postoperatively, two bladder specimens showed carcinoma cells, and a small tumor which had not been observed on previous cystoscopic examinations was found near the ureteral orifice. The catheter, in being introduced into the ureter, had picked up cells from this tumor and when they were found in the ureteral specimen it was naturally assumed that they came from higher up in the urinary tract. A similar instance of contamination of a specimen by cells from another site occurred in a gastric aspirate. Malignant cells were found which were believed to be of gastric origin, but further exploration proved that they were from a carcinoma of the lung. Review of the smears showed that intermixed with the malignant cells there were histiocytes containing particles of carbon which are commonly found in sputum.

In procuring gastric specimens, preparation of the patient plays a very important part. The stomach must be free of any food or foreign material which will interfere with interpretation of the cells. Under ordinary circumstances, the stomach will be fairly clean in the morning if the patient has had a liquid evening meal, and no food, liquids or medication by mouth after midnight. In a case of gastric retention, however, the patient should be kept on a liquid diet the entire day prior to the test, and continuous gastric aspiration the night before might be required in order to thoroughly cleanse the stomach. After a gastro-intestinal series, barium is retained in the stomach for a variable length of time, so it is always desirable to wait at least three days before obtaining a specimen for cytologic study.

Because of the digestive action of the gastric juices on exfoliated cells which have collected in the stomach, the number of well preserved cells which can be recovered by ordinary aspiration is often insufficient. A greater number can be obtained by using the gastric balloon[1] which, because of its mildly abrasive action, dislodges cells from the gastric mucosa. Such cells are better preserved due to their recent exfoliation and usually represent a greater area of the lining of the stomach than do naturally exfoliated cells. The balloon, covered with wide-meshed hat veiling, is attached to the end of a double lumened rubber tube. One lumen, used for aspirating, has a Rehfus bucket tip at the distal end and a 20 cc. syringe at the proximal end. The other lumen, used for inflating the balloon, is perforated in that portion which lies within the balloon, and has an inflating bulb attached to the proximal end. The deflated balloon is swallowed and after the stomach has been emptied by aspiration and thoroughly lavaged with Ringer's solution, the balloon is inflated. It is carried by peristalsis toward the

pylorus, deflated to allow its further passage into the antrum, re-inflated and gently pulled back to the cardia, thereby applying gentle friction to the entire lining of the stomach. This procedure is repeated five or six times, the gastric fluid being aspirated meanwhile at frequent intervals. A portion of each aspirate is saved and mixed immediately with an equal volume of 95% alcohol. The balloon is finally deflated, withdrawn and rinsed by vigorous shaking in a beaker containing equal parts of Ringer's solution and 80% alcohol. Any fragments of tissue clinging to the balloon netting are added to the rinsings. The aspirate and rinse specimens should then be sent immediately to the laboratory for centrifugation and preparation of smears. If a patient has had recent gastric bleeding or is known to have esophageal varices, the gastric balloon should not be used. A minimal amount of bleeding is sometimes produced by use of the balloon, but in our experience no hemorrhage has resulted from its use in over 500 cases.

In collecting rectal or colonic washings,[1] the presence of fecal material in the specimen is obviously to be avoided. The patient should be instructed to take two ounces of castor oil the preceding night and a warm soap suds enema in the morning. Two to five hours after the enema, saline washings are obtained and mixed immediately with 95% alcohol.

Specimens should be delivered to the laboratory without delay so that smears may be prepared before degenerative changes occur. Promptness is especially important where material from the gastro-intestinal tract is concerned, since deterioration takes place rather rapidly. If delay is unavoidable, specimens should be kept in the refrigerator until delivery can be made.

II. Laboratory Procedures:

All fluid specimens should be centrifuged as soon as possible and the supernatant liquid decanted to prevent degeneration of the cells. If smears cannot be prepared immediately, the sediment is covered with a few cubic centimeters of absolute alcohol and placed in the refrigerator. Sputum need not be centrifuged but must be examined for blood tinged particles which should be selected for smearing.

The technic of preparing smears from sputum and sediments is not a difficult one to learn, but does require some practice. The material must be spread evenly, and not so thick that the cells are in heavy masses, nor yet so thin that they are dispersed over too great an area, making the microscopic examination a tedious process.

One of the greatest difficulties in the preparation and staining of sediment smears is in making the material adhere to the slides. Not only is the loss of material a thing to be avoided, but the danger of contamination by "floaters," i.e., cells which become detached from one slide and stick to another, is a serious matter

which can lead to false positive reports. Several precautionary measures may be suggested for reducing this hazard to a minimum. Two or three drops of Mayer's albumen may be mixed with the sediment in the centrifuge tube[3], and slides are coated with a thin film of albumen to provide an adhesive surface for the smears. The sediment is spread uniformly on the slide, using the flat surface of another slide. The smear should be immersed very gently in the fixing solution of ether and 95% alcohol, and left there for at least one hour. If the smear is of a liquid consistency and shows a tendency to run off when the slide is tilted, it should be kept out of the fixative until it becomes tacky but not dry.

Before staining, the slides are transferred, without drying, from the fixative to a 1% solution of celloidin in ether-alcohol, for about a minute. They are then run through the descending alcohols, starting with 80%, and stained by the Papanicolaou method.[1] The celloidin forms a protective coating over the smear but does not interfere with the staining of the cells. Slides should be handled carefully throughout the staining procedure and should not be vigorously agitated in the various rinsing and dehydrating solutions. It is preferable to lower them gently into the jar and leave them there for a minute or longer until the smears are penetrated by the solution.

When staining urine sediment smears which are most prone to wash off, the usual Papanicolaou method can be modified slightly to avoid leaving them in running water, which is the usual procedure for differentiating the nuclear stain. The modified technic is to stain them in hematoxylin for two minutes, rinse in distilled water, then 50% alcohol, place for 1 minute in 1.5% ammonium hydroxide in 70% alcohol, rinse in 70%, 80%, and 95% alcohols and proceed with the counterstains in the usual way.

Gynecological smears, because of their mucus content, adhere to the slides much more readily than do sediment smears. It is therefore unnecessary to coat them with celloidin, and it is preferable to dip them in and out of each dehydrating solution several times in order to thoroughly penetrate the cells which may be covered with mucus.

Stains and rinsing and dehydrating solutions should be filtered frequently to keep them free of debris. Last but not least, when mounting slides with cover slips, one must guard against the transfer of cells from one slide to another, which is quite possible if the rod or dropper used for applying the mounting medium touches the smear. This can be avoided by dropping the mounting medium on the coverslip rather than on the slide.

Good staining technic is equal in importance to proper collection and fixation of the specimens and preparation of the smears. In the diagnosis of malignancy by exfoliative cytology, a great deal of emphasis is placed on the structure and staining properties

of the nuclei and nucleoli, and on certain cytoplasmic characteristics. Any hematoxylin which stains the nuclei well may be used but must be carefully controlled, since overstaining may give a false impression of hyperchromasia, one of the criteria of malignancy. On the other hand, if nuclei are understained, the chromatin structure is indistinct and malignant cells may be overlooked. As for the cytoplasmic counterstains, if the Papanicolaou method is used, a good balance should be maintained between basophilia and acidophilia, and the Orange G stain must be kept up to the proper strength so that highly keratinized cells such as those often exfoliated from epidermoid carcinomas will show the characteristic brilliant orange color. The careful technologist, therefore, keeps a watchful eye on all stains, reinforcing or renewing them as often as necessary. If the stains are fresh and correctly balanced, but the smears are poorly stained, the 95% alcohols used for rinsing after the counterstains may need changing more frequently, or the absolute alcohol or xylol may contain moisture so that the cells are not properly dehydrated and cleared. If the xylol or mounting medium are not neutral, the stained preparations may become decolorized. All excess xylol should be drained from a slide before mounting it with a coverslip to avoid any fading of the stains.

It should not be necessary to mention the importance of correct labelling of all specimen bottles, centrifuge tubes and slides so as to avoid any mix-up, not only of specimens of different patients, but also of multiple specimens from one patient. In some cases the clinician may depend to a large measure on the cytologist to locate the site of a tumor. For instance, if a lesion is suspected in the urinary tract but the exact location cannot be determined, specimens may be submitted from the bladder, and right and left ureters, and any error in labelling such specimens might result in very serious consequences.

It might appear from all the warnings and precautions just described that exfoliative cytologic technics are cumbersome and complicated. If analyzed, however, it will be seen that they do not add appreciably to the time necessary for carrying out the procedures and could easily become routine. The amount of extra time required to perform the tests correctly is negligible compared with the loss of time to the doctor, the patient and the technologist if the entire process must be repeated. Also, one must consider the discomfort to the patient if cystoscopic, bronchoscopic or gastric specimens have to be obtained again.

The value of exfoliative cytology as a method of cancer diagnosis when used as an adjunct to other diagnostic procedures has been proven and accepted; but, if it is to be relied upon, the smears submitted for evaluation must be of the best possible quality.

REFERENCES

1. Papanicolaou, G. N. Atlas of Exfoliative Cytology. Commonwealth Fund, New York. (Published by Harvard University Press, Cambridge, Mass.) 1954.
2. Ayre, J. E. Selective cytology smear for diagnosis of cancer. Am. J. Obst. and Gynec., 53: 609-617. 1947.
3. Staff of the Vincent Memorial Laboratory of the Vincent Memorial Hospital. The Cytologic Diagnosis of Cancer. W. B. Saunders Co., Philadelphia. 1950.

INACCURACIES PREVALENT IN ROUTINE URINALYSES*

By HELEN WIOT, M.T. (ASCP)

Milton Hospital, Milton, Massachusetts

It would be an ideal situation if everyone who hears or sees these words could honestly feel they need not be written, but I have seen a widespread tendency among well-informed and otherwise careful workers to minimize the need of accuracy in urinalyses. It is my purpose to note some of these careless techniques with a few observations on possible results.

First, in your hospital are you sure the specimens come to you in clean and correctly labeled bottles? Nursing personnel frequently feel that the performance of urinalyses is disagreeable, usually relegated to the least important person in the laboratory and therefore not of much importance. It is of utmost importance that the containers be scrupulously clean so that you may be absolutely sure that the sediment comes from the patient, not the bottle. This is especially true when reporting the presence of motile bacteria. Likewise if the busy delivery room nurse isn't quite sure of the patient's name the laboratory must take the responsibility of tracing the specimen before the report is final. The laboratory should also insist that catheter specimens are always so marked.

In the matter of description, each laboratory may have its own standards, but each worker should conform to the terminology of that laboratory so that one specimen won't be described in a variety of ways, depending on who is doing urines that day. It is also necessary that the doctors who see the reports are aware of the meaning of descriptive terms, especially in the matter of describing quantities of a material present, as in the case of albumin and sugar. I will discuss this more in detail later.

* Read before ASMT convention, June, 1954, Miami Beach, Florida.

I once knew of an old doctor who drove twenty miles to check his urinometer because he had taken a specific gravity of 1.018 which was reported on the same specimen from a public health laboratory as 1.020. This is probably leaning over backward in care, but in my own laboratory I found that all of the micro-urinometers were inaccurate and some were off by as much as 0.010. They would have been misleading in a concentration test, and yet when I called the supply house to order others I was told micro-spindles are usually inaccurate. They are, however, in fairly widespread use. The fact that a high albumin content will give an inaccurate specific gravity, which should be corrected, is often overlooked, and this is of especial importance in a concentration test.

If the pH of a specimen is to be reported accurately it is necessary that the color comparison be made carefully and that the test paper be kept in a container which will not permit contamination, also that it not be contaminated with the fingers in removing. A strongly alkaline specimen would always be checked to see if it is sufficiently fresh to warrant an analysis. If it has been sitting in the utility room for a day or two before someone thought to send it to the laboratory it should be discarded and another sample requested.

If screening tests for sugar and acetone are used confirmatory tests should be run on all positives. The powder screening tests are rapid but are supersensitive. And in the case of acetone frequently a drug will give a positive. Since this gives a positive in the confirmatory test also a little investigation is needed.

The less common tests appear to be done with somewhat greater care, proving that it is monotony of repetition which is causing the careless habits noted. Even so there are workers who are careless about making sure that all albumin has been removed from a specimen before it is tested for Bence Jones protein, and in ascertaining that all bile has been removed before a test for urobilinogen is done.

In the matter of reporting amounts of sugar present there is less variance since the amounts of precipitate in a Benedict's solution can be fairly well estimated, but albumin is reported in a wide variety of ways. If sulfosalicylic acid is used to determine the protein in urine, the Kingsbury-Clark standards may be used for comparison and a quantitative estimate may then be given. This is of real importance to doctors who must consider reports from several laboratories on a patient who may have early kidney disease.

Good quantitative technique is also desirable in reporting on urine sediments. Leucocytes and erythrocytes should be reported per high power field and a cover slip used even though cover slips are a bit difficult to polish and troublesome to use. Since a dia-

betic patient has to be watched for kidney in ction, this care in estimating the number of leucocytes is important. The custom in some hospitals of having the descriptive work and the chemistry done in one room and then having the sediments read elsewhere by a worker who doesn't see the rest of the report is not conducive to good work, nor is the separation of departments to such an extent that the worker who does urinalyses has little chance to learn the blood chemistry findings on the same patient. Even in obtaining the sediment there is room for careless technique. It is advisable that all specimens be centrifuged under standard conditions. Five minutes at 1500 R.P.M. is usually recommended. And care should be taken that a representative portion of the sediment be transferred to the slide.

Don't hope you were correct about red cells versus white cells when a drop of acetic acid would make you sure.

Don't forget to familiarize yourself with common artefacts.

Don't forget there is a quick easy test for the sulfa drugs.

Don't forget to look for red cells when calcium oxalate crystals are present.

Don't underestimate the importance of the lowly urinalysis.

REFERENCES

1. Approved Laboratory Technique—Kolmer and Boerner, p. 121.
2. Approved Laboratory Technique—Kolmer and Boerner, p. 150.
3. A Syllabus of Laboratory Examinations in Clinical Diagnosis—Thomas Hale Ham, p. 250.

METHODS AND NOMENCLATURE FOR 17-KETOSTEROIDS*

By ANNA L. RUNDELL
Jackson Memorial Hospital, Miami, Florida

The 17-ketosteroids have in common with all steroids a "cyclopentanoperhydrophenanthrene" nucleus, but derive their name from the presence of a ketone group at the C-17 position. It is the presence of this group upon which the color reactions are based. The neutral 17-ketosteroids are degeneration products of testosterone and corticosteroids; the former going to androsterone and etiocholane while the latter forms these two as well as dehydroisoandrosterone and isoandrosterone. In these products, it is the position of an hydroxyl group at C-3 which determine whether the steroid is alpha or beta. In the alpha, the OH is below the plane of the molecule and in the beta, it is above. Under normal circumstances androsterone and etiocholane, which are alpha, are present in much larger amounts. The beta fraction is made up largely of isoandrosterone and dehydroisoandrosterone which are of the beta configuration.

The urinary 17-ketosteroids are products of the adrenal cortex in women and of the adrenal cortex and testes in men. Variations in the amount excreted especially in certain pathologic conditions are in some measure reflections of changes in the amounts of steroids produced by these two glands.

In setting up a method for the determination of 17-ketosteroids, we were faced with the same problem that you are. We wanted a method which was as accurate as possible but not requiring any more time than necessary. The Girard's Reagent T for separation of the ketonic fraction from the non-ketonic was discarded because of the time required and the lower value due to unavoidable loss. Two methods, both modifications of the Holtorff and Koch procedure, were compared on people of differing age and sex. Since the method of correcting for the presence of chromogenic material by reading the unknowns at both violet and green wavelengths can not be used with the Holtorff and Koch method, these modifications make use of two other compensatory mechanisms. One ran a blank on the urine extract, color determination on the urine itself, and a color determination on the urine plus the standard. Also, a standard was run, and blanks on the reagents as well as on the alcohol and potassium hydroxide without the color reagent. The other method extracted the test solution with chloroform after development of color and dilution with 60% alcohol. The chloroform contained the red pigment which is characteristic of 17-ketosteroids and left the brown color in the aqueous alcoholic phase. Only a reagent blank is needed. Since the chloro-

*Read before ASMT, June, 1954, Miami Beach, Florida.

form color extraction method of Cahen and Salter checked with the other method and was somewhat simpler, we adopted it.

All the tests are done on aliquots of 24 hour samples to which 4-5 ml. of concentrated hydrochloric acid has been added. If it is to stand over 24 hours usually toluene is added. The acidified urine may be stored in a refrigerator for several days without loss of 17-ketosteroids. If the patient has diabetes insipidus, it is well to restrict fluid intake during the collection time. In the Cahen and Salter method after acid hydrolysis, two ether extractions are used. The ether extracts are combined, washed once with 10% sodium hydroxide, and once with distilled water. Then the ether extract is made to the initial volume of the urine aliquot started with in the hydrolysis, par ex. 25 ml. of urine hydrolyzed, so the final volume of the treated extract is 25 ml. Five milliliters of this volume is evaporated with heat and suction. To the residue add 0.2 ml. of absolute ethyl alcohol, 0.2 ml. of m-dinitrobenzene, and 0.2 ml. of 5 N potassium hydroxide. Mix between each addition and start timing from the addition of the hydroxide. Color development is in the dark at 25° C for 1 hour. When the tubes are removed, 5 ml. of 60% ethyl alcohol and 5 ml. of chloroform are added. This is well mixed, placed in an ice bath for 3 minutes, centrifuged for 3 minutes, and then read at 520 mu against a reagent blank. The test is set up in Coleman tubes and can be read immediately after centrifugation. Color development proceeds for two minutes and fades after 20 minutes, so by working rapidly fading becomes negligible.

One disadvantage to the method is that the results run somewhat high. Our recovery experiments ran about 108%. The test can be left at the ether stage, but leave the extract evaporated in the Coleman tubes in a desiccator. The results will be somewhat lower if left overnight in solution even if the ether solution is placed in the refrigerator. The potassium hydroxide, in a polyethylene bottle, m-dinitrobenzene, and diluted standard are kept in the refrigerator and removed one-half hour prior to use.

If the values are low as in children, old people, or adrenal insufficiency, 10 ml. may be evaporated to run the test. The results are not exactly linear but 1 mg./24 hr. has been our greatest deviation which is not clinically significant. Since the amount of color developed with a given amount of 17-ketosteroids changes gradually as the reagents age, it is desirable that a standard be included in any series of determinations. The normal values for 17-ketosteroids, besides depending upon the method used, also vary with the age and sex of the subject.

REFERENCES

1. Charles H. Sloan and George H. Lowrey. Endocrinology, **48**, 384 (1951).
2. A. F. Holtorff and F. C. Koch. J. Biol. Chem., **135**, 377 (1940).

3. Raymond L. Cahen and William T. Salter. J. Biol. Chem., **152**, 489 (1944).
4. Rabbie and Gibson. J. Clin. Endocrinol. Metab., **3**, 200 (1943).
5. R. S. Harris and K. V. Thimann. Vitamins and Hormones, Several Volumes, 1943 and following, Academic Press, Inc., New York.
6. G. Pincus, Recent Progress in Hormone Research, 1947, Academic Press, Inc.
7. J. Masen, personal communication.

THE DETERMINATION OF URINARY 17-KETOSTEROIDS*

By DR. JOHN McANALLY
University of Miami School of Medicine, Miami, Florida

There are two principal problems in routine determinations of 17-ketosteroids in urine. The first is loss and destruction during hydrolysis, the second is elimination of or compensation for non-specific urinary pigments. Neither difficulty is an insurmountable one, but it must be realized that when the usual methods are employed the analysis for 17-ketosteroids is at best a semi-quantitative estimation.

The 17-ketosteroids of urine are excreted as conjugates, either as sulfates or as glucuronidates. The accepted procedure is the hydrolysis of these conjugates by refluxing the urine sample for ten minutes with one-tenth its volume of concentrated hydrochloric acid. Some destruction takes place under these conditions, but the products still react to give a color with the commonly used reagents. The acid should not be added until the urine has come to a boil, and when the reflux period is completed the sample should be cooled in an ice-water bath. Enzymatic hydrolysis shows promise of being an excellent research tool, but the time requirements make its routine use unadvisable.

Extraction of the hydrolyzed urine can be carried out with ether or with carbon tetrachloride. Ether must be peroxide-free if it is to be used. The use of ether has certain advantages and and disadvantages. Ether has a greater tendency to form emulsions, but it is less toxic and its lower boiling point makes its disposal less of a problem. Carbon tetrachloride can be added to the reflux flask for continuous extraction, but requires more precautions because of its toxicity. A well-functioning hood is necessary.

The non-specific urinary pigments which accompany the 17-ketosteroids have given rise to several special methods which seek to remove the interference before the application of the final color raction. Girard's Reagent T will effect a separation, and will remove not only the pigments but also any interfering

*Read before ASMT, June, 1954, Miami Beach, Florida.

non-ketonic steroids present. This procedure is time consuming and can be used only by a well-trained individual. Losses of 17-ketosteroids are inevitable, and even with careful attention to detail will be about 15%. Zimmerman has used charcoal filter paper to remove the color, but the author has had little success in repeating that work. A unique method has been developed recently by Drekter, et al, in which the color is removed by treatment of the crude ethylene dichloride extract with solid sodium hydroxide. This method, with a later modification, has not been evaluated here but it seems promising for clinical purposes.

Color development with m-dinitrobenzene is a much studied process, but little agreement has been reached as to the best reagents. Potassium hydroxide solutions in water, ethanol and methanol have all been advocated. Any one of the three is good if the proper precautions as to stability are observed. The methanolic solution is stable; the ethanolic is not. The potassium carbonate which forms and dissolves in the aqueous solution may prove troublesome unless 60% ethanol is used for the final dilution. The carbonate will precipitate with 95% ethanol. The use of antimony trichloride dissolved in acetic anhydride has been suggested as a reagent. The disadvantage is that dehydroepiandrosterone gives a very much less intense color than do the other common urinary 17-ketosteroids.

It should be stressed that 17-ketosteroid analysis with a degree of accuracy compatible with clinical requirements is feasible for any well-trained technician. The equipment and reagents needed are neither expensive to obtain nor difficult to use. Careful attention to detail will result in very satisfying results.

REFERENCES

1. I. J. Drekter, A. Heisler, G. R. Scism, S. Stern, S. Pearson and T. H. McGavack. J. Clin. Endocrinol Metab., **12,** 55 (1952).
2. H. Pfister. Biochem. Zeit., **323,** 157 (1952).
3. G. Pincus. Endocrinology, **32,** 176 (1943).
4. Recent Progress in Hormone Research, v. IX, 1954, Academic Press, Inc.

THE GAVEL

In a beautiful and inspiring book written by Antoine de Saint-Exupéry, the following quotation appears as part of his instructions and advice to teachers. "You shall not fill them with hollow formulas but with visions that are portals of creative action."

These simple yet magnificent words, I believe, can be an appropriate expression of the goal—the ideal of professional life whether it be manifested in everyday work, in teaching, or in society and community activities. The words are dynamic. They imply movement and energy, giving and taking, stimulation and inspiration. Each can interpret them according to his own dreams and ambitions. Each can apply them according to his own principles and ethics.

The coming months will bring many local meetings and seminars as well as the annual national convention. Examine issues critically and intelligently without personal prejudice. Contribute to growth of scientific knowledge energetically and willingly. Then truly for each of us will open many "portals of creative action."

RUTH HOVDE

OBITUARY

The death in February of Miss Edna Murmann, Executive Secretary of the Illinois Association of Medical Technologists and member of the editorial staff of this journal is a great loss to medical technology. Her leadership and great courage will continue to be inspiration to all those who knew and worked with her.

ABSTRACTS

SEPARATION OF INDIVIDUALS OF ANY BLOOD GROUP INTO SECRETORS AND NON-SECRETORS BY USE OF A PLANT AGGLUTININ (LECTIN)

William C. Boyd and Elizabeth Shopleigh. (Boston University School of Medicine). Blood 9, 1195-7 (1954).

An anti-H agglutinin prepared by simple saline extraction of seeds of Ulex europeus was found suitable for routine separation of individuals into secretors and non-secretors. This was done by testing the ability of undiluted saliva to inhibit the agglutination of group O erythrocytes by the undiluted Ulex extract. The extract was made by mixing the seeds ground to meal in a mill, with ten times their weight of 0.9% saline, in a shaking machine for an hour centrifuging and filtering. The stock solution was kept in the deep freeze and thawed as needed.

Equal quantities (0.05ml) of the Ulex extract and undiluted, boiled, centrifuged saliva were mixed and allowed to stand 30 min. at room temperature. Then 0.05 ml of a 1% suspension of group O cells were added, and the mixture mixed, centrifuged and read with shaking. If no agglutination of the RBC occurred the result was recorded as inhibition and if agglutination took place the result was recorded as no inhibition. The symbol (Se) means secretor, (se) non-secretor .

An advantage of requiring only one reagent for salivas from individuals of any blood group and only one type of erythrocutes for testing was shown. There was no disagreement between the diagnosis made in the usual way and that based on using Ulex extract and group O cells.

SPIROCHAETOIDEA AND PROTOZOA PATHOGENOUS FOR MAN AS SEEN IN FIXED UNSTAINED SMEARS UNDER DARK-FIELD ILLUMINATION OR THE PHASE CONTRAST MICROSCOPE

Edna Silva-Inzunza, Waldemar E. Coutts and Waldemar R. Coutts. (University of Chile School of Medicine). J. Trop. Med. and Hyg. 57, 292-4 (1954).

The fixed, motionless micro-organisms can be studied under different power lenses in the same field. A thin smear of the suspected material is made and, before complete drying at room temperature, it is covered with 10% formol solution or with Bouin's fluid for ten minutes, washed gently under tap water and dried between filter paper. Such preparations keep for months, can be collected anywhere and sent to the laboratory for diagnosis. Flagellae and cilia can be seen far better than when stained.

A METHOD OF SECTIONING FOR THE ELECTRON MICROSCOPE

Delbert E. Philpott. (Marine Biological Laboratory, Woods Hole). Exp. Med. and Surg. 12, 445-9 (1954).

Simplicity and speed are claimed with the method written for use with a conventional spencer 820 microtome and a glass knife holder. A bowl-shaped boat of 15 ml. capacity made of dental acrylic is formed to fit the glass knife.

The embedding plastic is mixed ⅔ butyl to ⅓ methyl methacrylate. Isopropyl alcohol is used for dehydration. The capsule-shaped plastic containing the specimen is turned into a screw thread hole which keeps it rigid. It is then advanced to the cutting edge in increments of one micron until assurance is reached that the specimen is being sectioned. If the specimen is passing the knife without being cut gentle pressure is applied with a tongue depressor to the back of the knife holder each time a cutting stroke is made. The sections are collected directly from the boat on 400 mesh copper screens and placed on a paper towel so that the liquid is drawn through the screen and the section pressed firmly onto the screen by surface tension. Methods of Hillier and Gettner were used for fixing and staining (Science 112, 520-3, 1950).

SERIAL SECTIONS FOR ELECTRON MICROSCOPY

Helen Gay and Thomas F. Anderson. (University of Pennsylvania) Science 120, 1071-3 (1954).

Serial sections only a few hundred angstroms thick are possible for electron microscopy with new microtomes. Unknown cellular structures in three dimensions may be reconstructed. Ribbons are picked up with a Formvar-coated wire loop and placed over the slits in Sjöstrond-type specimen holders (obtained from Smethurst, High-Light, Ltd., Sidcat, Lancs., England). Tissue is fixed in 1 per cent buffered osmium tetroxide and imbedded in n-butyl methacrylate. The block is trimmed under the dissecting microscope as close as possible to the desired cells, to leave a surface rectangle about 0.3 by 0.08 mm. With the long dimension parallel to the knife edge a straight ribbon is usually obtained. When 15 to 25 sections are cut it is detached from the knife edge. A trough whose liquid level can be controlled by the manipulation of a hypodermic syringe connected by a plastic tube to an opening in the base of the trough will aid in detaching and floating sections. The level of the liquid is raised above the knife edge so as to have a well-rounded meniscus and the ribbon is detached with a fine hair and floated from the shallow liquid near the knife edge to the deeper part of the trough.

A wire loop (about 4 mm in diameter) made of 0.006 in. "Advance" wire (43% nickel, 57% copper) supporting a thin film of dried Formvar is placed into the liquid of the trough at a 45- to 90-degree angle and the ribbon is centered across its diameter. When the loop is raised slowly from the trough, the sections adhere to the Formvar and are transferred to the supporting grids in the electron microscope for examination.

A transparent plastic assemble designed to fit the condenser mount and to support a Sjöstrand-type grid on top of a Lucite rod ⅛ in. in diameter and a circular disk, 10 cm in diameter with a central opening 10 mm in diameter, which rests on the microscope stage and supports the loop holding the sections. The loop is attached to the circular disk and placed in the central opening. The section ribbon is then optically superimposed over a slit in the grid, the condenser mount raised to bring the grid and Formvar film into contact. Further elevation causes the rod with the grid to pass through the wire loop. In this manner the Formvar film including the sections located over one of the slits of the grid, is firmly attached to the surface of the grid and is ready for examination in the electron microscope.

Electron micrographs of serial sections (18) cut with a Porter and Blum microtome of the salivary gland cell of a larva of D. melanogaster, representing a total thickness of less than 2 microns, demonstrate the potentialities of using serial sections for determining three-dimensional distribution of cytoplasmic structures such as the endoplasmic reticulum and secretion granules.

THE CYTOLOGICAL BASIS FOR THE ROLE OF THE PRIMARY DYE IN THE GRAM STAIN

Carl Lamanna and M. F. Mallette. (Johns Hopkins University School of Hygiene and Public Health). J. of Bact., 68, 509-13 (1954).

Specificity of triphenylmethane dyes as primary dyes in the gram stain rests on the unusual ability of these dyes to stain the cell wall of gram positive organisms and their inability to stain the cell wall of gram negative organisms. As a satisfactory primary dye they form a colored, water insoluble lake upon mixing with a mordant solution containing iodine.

Counterstaining with certain organic solvents removes the lake from the cell walls of gram positive organisms because of the solubility of the lake in the organic solvent. The most effective staining of gram positive forms took place at alkaline pH values without washing between the application of primary dye and mordant. When washed, at a low pH the dye disappeared from the walls. Organisms appear gram positive when their stained walls resist both decolorizer and counterstain after treatment with iodine.

DIFFERENCES IN SUSCEPTIBILITY OF POLYMORPHONUCLEAR LEUCOCYTES FROM SEVERAL SPECIES TO ALTERATION BY SYSTEMIC LUPUS ERYTHEMATOSUS SERUM: APPLICATION TO A MORE SENSITIVE L. E. PHENOMENON TEST

Ana E. Carrera, May Virginia Reid and N. B. Kurnick. (Tulane University School of Medicine) Blood, 9, 1165-71 (1954).

L.E. serum used against leucocytes from 12 different animal species showed L.E. cells "globs" (free homogeneous basophilic masses), droplets and rosettes per 1000 white blood cells and per 1000 polymorphonuclear cells after 2 hours incubation. Controls were similar preparations using normal human serum and homologous plasma. The chicken and horse were most susceptible while man was low in susceptibility. The use of chicken, horse, guinea pig or dog leucocytes instead of human leucocytes is believed to give a highly sensitive L.E. test.

Venous blood with heparin as the anticoagulant was obtained from twelve animal species. Smears were made immediately and the blood centrifuged at 2000 r.p.m. for 10 minutes and the plasma and buffy coat were separated from the red blood cells. The leucocyte suspension was divided into three portions in 10 X 100 mm. test tubes, centrifuged at 2500 r.p.m. for ten minutes, the supernatant decanted and the sediment (WBC button) treated in this manner: one WBC button resuspended in 0.35 ml. of normal human blood serum, another button resuspended in 0.35 ml. of homologous plasma, and the third resuspended in 0.35 ml. of L.E. serum. The same L.E. serum and the same control serum were used throughout the experiment. Sodium ethyl-thio-mercurisalicylate ("Merthiolate," Lilly, final concentration 1:100,-000) was added to the fresh sera and the sera kept at —20 C until used.

The three preparations of WBC suspensions were incubated at 37 C in a waterbath for two hours, centrifuged at 2500 r.p.m. ten minutes and smears prepared from the sediments. After staining with Wright and Giemsa stains they were examined under oil immersion.

The smears of whole blood were examined for cell morphology and to determine the normal differential distribution of leucocytes of the given animal species. Those preparations incubated with normal human blood serum and homologous plasma served as controls. The L.E. preparations were studied as follows: (1) 1000 white blood cells of all types were counted with simultaneous recording of any L.E. manifestation (L.E. cells, rosettes, droplets, and homogeneous free basophilic masses, "globs"); (2) similar recording per 1000 polymorphonuclears. In such counts, a "glob" was counted as a polymorphonuclear cell.

ABBREVIATION OF BACTERIAL GENERIC NAMES

S. F. Cowan. (National Collection of Type Cultures, London). Science, 120, 1103-4 (1954).

Analysis of replies to questionnaire to 77 English language journals concerning abbreviation of bacterial generic names failed to show any significant difference in the views of editors in different countries; nor in journals devoted to medical or non-medical sciences. Medical journals gave more freedom to authors but about half of them issue instructions or suggestions for authors to follow. Several stressed that clarity is more important than space-saving. The generic abbreviations given in Darlands "Medical Dictionary" are used by several American medical journals.

Four possible solutions are presented for consideration:

1. Paragraph. The generic name is written in full the first time it appears in a paragraph and its initial only (if no other genus is used having the same initial) when mentioned in the same paragraph.

2. Page. The same principle as in #1 is applied to a page instead of to a paragraph.

3. Paper. The same principle as in #1, that is, the full name when first mentioned. (a) in the title, (b) in the paper and (c) in the summary with the subsequent use of an initial letter for the same generic name. Where ambiguity might occur the name would be completely written.

4. Do not use abbreviations of generic names, except when a list of species of the same genus is used, in which case the initial letter would be taken.

CHROMOGENIC BACTERIA WITH A CASE REPORT OF A FATAL INFECTION CAUSED BY SERRATIA MARCESCENS

Robert G. Vernon and Opal E. Hepler. Quart. Bull. Northw. Univ. Med. School 28, 366-72 (1954).

This paper brings together case reports and experimental data regarding pathogenicity of the chromobacteria; it reports a case of fatal infection due to Serratia marcescens and compares the bacteriologic characteristics of certain chromogenic bacteria.

Chromobacterium violaceum was quite evidently the etiologic agent in three cases. In the first two and probably in the third, the primary infection was caused by this agent. Prognosis is grave in infection caused by Chr. violaceum.

Serratia marcescens. Its pathogenicity was demonstrated for laboratory animals in 1903. In man it may be a primary bacterial invader in debilitated patients or it may become a secondary bacterial invader of organs previously infected by other organisms. It is completely resistant to all antibiotics except, perhaps, neomycin. Death from sepsis results if the organism becomes disseminated.

POTENTIAL INFECTIOUS HAZARDS OF LABORATORY TECHNIQUES

Morton Reitman et al. (Camp Detrick, Frederick, Maryland). J. of Bact. 68, 541-52 (1954).

I. Lyophilization

The light fluffy or hard claylike material of dried organisms in a highly concentrated form presents a hazard to the laboratory worker. This paper treats of the contamination possible throughout the lyophilizer. The use of double-trap cotton filter prevented the contamination of the vacuum gauge and pump and allowed the safe removal of the remainder of the apparatus for sterilization.

II. The Handling of Lyophilized Cultures

Contamination of the environment produced by handling of lyophilized material presents a hazard to laboratory workers. A considerable aerosol is produced when an ampule is opened in the usual way and large numbers of organisms are set free into the surroundings. This was greatly reduced by surrounding the point of ampule breakage with a 70% ethanol soaked cotton pledget. Reconstitution of lyophilized cultures by three diferent methods produced aerosols.

Decontamination with 2% lysol and ultraviolet light, and the use of an exhaust system reduced air contamination. The greatest margin of safety is the use of a bacteriological safety cabinet.

III. Viral Techniques

Contamination of the rubber diaphragm of the vaccine bottle when making dilutions of infectious materials was greatly reduced by using an ethanol soaked cotton pledget around the needle and swabbing the diaphragm with alcohol. Extensive contamination and aerosols were produced by the intranasal inoculation of mice. Its solution seems to be the use of a ventilated cabinet to contain the organisms and ventilated cages to house the inoculated animals.

For intracerebral inoculation the safest method was the use of an ethanol soaked pledget around the needle together with decontamination of the injection site with 2% tincture of iodine. Shell contamination was considerable when allantoic and yolk sac inoculation of embryonated eggs was made. This was greatly reduced by the use of an ethanol soaked pledget around the needle. Inoculation of the chorioallantoic membrane by the window technique also had the possibility of shell contamination. Harvesting infected allantoic and amniotic fluids caused heavy surface contamination of egg trays, shells and hands of operator. Maceration of infected tissue in the Ten Broeck grinder produced aerosols. A protective ventilated cabinet is indicated when carrying out viral techniques.

OBSERVATIONS ON THE ANTIBODY CONTENT OF THE BLOOD IN PATIENTS WITH MULTIPLE MYELOMA

Herman A. Lawson et al. (Veterans Administration Hospital and Brown University, Providence, R. I.) *New Eng. J. of Med. 252. 13-8 (1955).

Nine cases of multiple myeloma were compared with normal persons and patients with other diseases as to the presence and titer of certain antibodies. A complete absence of antibodies or a marked deficiency of these substances is a characteristic feature of myelomatosis. The serum protein of five of the patients was analyzed by filter paper electrophoresis. Four showed marked increase in gamma globulin and the fifth a tall peak of the so-called M component but no gamma globulin. Four showed a complete absence of antibodies, including the isoagglutinins, and the fifth almost complete absence of antibodies and no isoagglutinins. This abnormality is considered to be the result of the abnormal function of the malignant plasma cells.

ANNOUNCEMENTS

COURSE IN THE CLINICAL PATHOLOGY AND LABORATORY DIAGNOSIS OF PARASITIC DISEASES

A short intensive course on the laboratory diagnosis and pathology of parasitic infections will be presented August 15-27, 1955, at the Louisiana State University School of Medicine in New Orleans.

The course is designed primarily for pathologists and technologists. However, general practitioners, internists, pediatricians, gastroenterologists and physicians engaged in the practice of public health and tropical medicine who are interested in the laboratory diagnosis of parasitic infections are welcome to attend. The instruction and training will be of assistance to pathologists who are preparing for board examinations, to pathologists and physicians who are responsible for the diagnosis of parasitic infections in their laboratories and to technologists engaged in this specialty.

The course will include lectures, extensive demonstrations, films and

supervised individual laboratory study. Emphasis will be placed upon the practical aspects of laboratory diagnosis of common parasitic infections, including training in stool examination and stool concentration technics. Abundant material from patients with parasitic diseases endemic in this area will be available for examination. Comprehensive slide sets containing parasitic organisms in tissue sections will be studied. Library facilities are available. The medical school building is air conditioned.

Registrants should bring their microscopes, equipped with mechanical stages, and their microscope lamps. A limited number of places will be available. The fee for the course is $50.00.

Persons interested in attending this course may write to:

Dr. Clyde Swartzwelder
Department of Microbiology
Louisiana State University School of Medicine
1542 Tulane Avenue
New Orleans 12, Louisiana

THORNDIKE MEMORIAL LABORATORY ANNOUNCES A COURSE IN HEMATOLOGY

A course in hematology will be given at the Thorndike Memorial Laboratory from June 6 through June 17, 1955. This same course will be repeated during the two weeks of June 20 through July 1, 1955. These courses offer advanced work in hematology to technologists, and pathologists who are familiar with the usual clinical laboratory methods.

The tuition fee is seventy-five dollars. Applications should be sent to Miss Geneva A. Daland, Thorndike Memorial Laboratory, Boston City Hospital, Boston 18, Massachusetts. Early application is essential as the number that can be accommodated is limited.

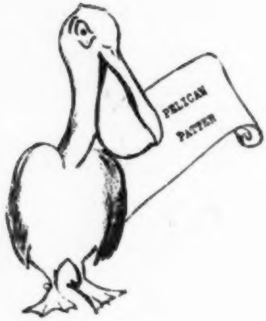
ANNOUNCEMENT

"The 32nd Annual Conference of the American Physical Therapy Association will be held at the Hotel Jefferson in St. Louis, Missouri, June 20-24, 1955."

ANNOUNCEMENT

The many friends of Mary Frances Gridley, whose death on December 31, 1954, was noted in the January issue of this journal, would like to dedicate a Special Edition of the Manual she compiled as a fitting and lasting tribute to her work. This would include her picture as a frontpiece and one or more appropriate eulogies. Since Miss Gridley used much copyrighted material, she had to agree that the Manual would never be sold. Therefore, a special printing would have to be distributed without revenue. It has been estimated that the type of publication that is planned will cost between one and two dollars per copy. A special fund, to be known as the Gridley Memorial Fund, is being established in order to acquire sufficient money to publish the Manual. Undoubtedly, many members of the American Society of Medical Technologists will desire to contribute to the fund. Please send contributions to:

Evelyn F. Ballou, MT (ASCP)
Armed Forces Institute of Pathology
Walter Reed Army Medical Center
Washington 12, D. C.



Eh, la bàs,—tous mes amis, —the time—she is about come, —and you will all be treckin down the ole Mississippi to La Nouvelle Orléans and the 23rd convention of the American Society of Medical Technologists—I say that long name correct, n'est-ce-pas?

We just going to say once more what we told you before —what a fine time we all going to have together—what you going to see and do and enjoy — No use a convention without some fun! Vraiment?

Now Sunday—what an introduction to our City—right away, you plunge in and make the acquaintance of that famous French Quarter—you see nothing more exciting nor foreign in Paris or even Madrid—and when you come back all exhilirated with old world charm and glamour—all ready to don your fancy clothes for the opening reception! I tell you—this is style, oui!

An then, Monday—a cocktail party—this in the fine, new—"moderne" quarters of the A. S. Aloe Co.,—Pretty, n'est-ce-pas, that name there—Aloe—like we say—Hallo down here—or maybe like Aloha—like in Hawaii —anyway—they give a beautiful party. And then on Tuesday you go to the dinner at the Court of the Two Sisters—Now that place you have to see to believe! I won't say a word about the crepe myrtles and the candlelight and the magnolias and sweet olive—not a word!

Then Wednesday—that ole man river—you can't get away from him—he keep going all around N.O.—and you get on the big old paddle wheel boat at noon for to see for yourself how great she is. You're going to pass a wonderful afternoon!

Now then—now—on Thursday—that's the day. That's when you going to meet **ME.** Right out in that Jung Hotel, yes—right in the banquet, there I'll be—and—you wait—when you come—you going to see and hear a big surprise!

And that is the formal end of the convention—but now wait—don't go running home yet—There's still more here for you to do. Even if you don't belong to—what they call it—the house of delegates—what kind of house that is, I wonder. There are still things planned. You ever heard of Carville, —the fine hospital which is making new all those poor people who suffer so bad from Leprosy—Hansen's Disease, we rather call it? There is a beautiful place like a college campus and it makes your heart happy to see how much can be done for these brave people. You know this is the only leprosarium in the whole United States? And here is your chance to see something of the famous old river road winding about along side the levee—because your bus will go up the river that way and back again—quick—on the super highway.

We feel ourself sad now. You will be going home—and we wring our hands in melancholy. All year we have been waiting and planning for you,—and all what will make us feel better will be to know you have passed a pleasant time and always will think nice of our Nouvelle Orléans.

Au Revoir,
Patate le Pelican.

SPECIAL LOUISIANA PATTER

Bienvenu à Louisiana. That's our way of saying "Welcome to Louisiana." With all of our traditionally known southern hospitality, we cordially invite "you all" to leave your cares at home and be with us at the 23rd Annual Convention of ASMT at New Orleans, Louisiana, June 12-17, 1955.

Since we are known as the "Pelican State," it is fitting that we should have selected a member of this feathered family to keep you informed of the "likely doings" in New Orleans. "Patate," the good-natured pelican with the big lugrubious eye from the deep bayou country, has already given you a gist of the unique attractions and rare charm that are in store for you. If you neglected reading "Pelican Patter" in your past issues of your ASMT Journal, it is suggested that you do, so that you may become familiar with the varied types of interest that Louisiana and New Orleans have to offer.

Patate, the French word for potato, was given to this particular pelican because of his rotund physique. He speaks with a "patois" (dialect) common to the small section of the bayou country in southwest Louisiana. This section is known as the Evangeline Country and its people are referred to as "Cajuns" a word derived from "Acadian." These Acadians—early French settlers in Canada—migrated to Louisiana from Nova Scotia exactly 200 years ago. Evangeline, heroine of Longfellow's epic poem, is enshrined in a small churchyard in the town of St. Martinville, La.

Those of you driving in to New Orleans from the west should allow a little time in seeing this beautiful bayou country so showingly described in Harnett Kane's, "The Bayous of Louisiana," "Deep Delta Country." We suggest that you take highway 90—what seems to be a sea of grass on both sides of the road (or train) from Lake Charles into Lafayette is rice. From Lafayette in to Raceland, you will see acres and acres of sugarcane, —still four months from cutting time. Just 9 miles north of New Iberia, on highway 25 or 9 miles outside of Lafayette take 479 you will find the little churchyard with Evangeline's grave. Seven miles south of New Iberia on highway 445 is Avery Island, famous for its vast E.A. McIlheny Bird Sanctuary, great salt domes and magnificent Jungle Gardens with rare plants from all over the world.

As you leave New Iberia, you will continue on highway 90, along the quaint, winding beautiful Bayou Teche. You will drive for miles along this picturesque bayou, seeing the great oaks laden with spanish moss, the cypress knees jutting out along the waters edge, the wild iris and water hyacinths and large white lilies. Watch for the exquisite white American Egrets! Now and then you will catch a glimpse of one of the beautiful colonaded mansions where once was centered much of the culture and ante-bellum social life.

Coming from the north you will find it very much worth your while to come through Natchez with its beautiful ante-bellum mansions, to St. Francisville then over to Baton Rouge. Here you will see the State Capitol, the South's tallest building which is reported to be the most beautiful state capitol in the nation. As you leave Baton Rouge, you may take the super highway (61) or you may follow the winding river road, equally famous for beautiful old homes and the buildings at Convent, now a Jesuit retreat house.

Coming from the east, highway 90 takes you along the sparkling Gulf Coast, as attractive to Northern visitors in winter as it is to our own people in summer.

Those coming in by air will be able to enjoy a panoramic view of the Crescent City while those coming by train will enjoy a view of our new Union Station and may even take time to review the beautiful murals on the walls of the waiting room.

Now in New Orleans—your first impression is that it is unique—you

French Quarter Street—New Orleans

will have a feeling that you are both in the United States and out of it. It is truly an American City, but more than any other American city, it offers a distinct foreign atmosphere.

On Sunday afternoon, we have planned a choice of two tours, the first for those with just a limited amount of time and an absolute "must": two hours through old New Orleans; and the other, taking a longer tour and contrasting and combining the modern city with the mellow beauty of the old section. To make sure you see one of the romantic courtyards for which New Orleans is famous and to be sure that you have opportunity to taste one of our world famous French Cuisines we have planned a dinner "under the stars" at the Court of the Two Sisters, in the heart of the Vieux Carre.

No trip to New Orleans is complete unless you see the second largest port in the U. S. So we have planned a trip on the S.S. President to give you a comprehensive picture of the majestic Mississippi and its bustling activity.

In the curtural realm, there will be opportunities for you to enjoy an outdoor summer "Pops" concert in Beauregard Square (formery Congo Square). It is here that the slaves used to dance to voodoo rites. If you are interested in art, a visit to Delgado Art Museum in City Park will be of interest. Dixieland jazz, characteristic of, and heard at its best in New Orleans, may be heard at various spots in the City.

Let's not overlook the gourmet's capital of the world. It is here that you may enjoy such dishes as Oyster Rockefeller, Pompano en Papillote, Poulet Rochambeau, Boullabaisee, Shrimp Arnaud, Crepe Suzettes, Café Brulot, etc., at their best—and—don't forget to enjoy the Original Sazerac —it's New Orleans' most famous cocktail. And when speaking of sea food, a visit to one of the many informal crab and shrimp restaurants out on the lake front means delicious food and an interesting three mile drive.

Don't for one minute think this is all there is to see and do in New Orleans. There'll be a detailed list of tours, famous eating places, night clubs, waiting for you at the Registration Desk.

And last but not least—we've got a grand surprise for the banquet which we promise will be as different and delightful as New Orleans is itself.

PROGRAM COMMITTEE PATTER

In the last issue of your Journal, we listed some of the outstanding speakers on the program and the subjects they will discuss. We would like to add the following:

I. Guest Speakers:

1. Dorothy Sundberg, Ph.D., M.D., Associate Professor of Anatomy, University of Minnesota Hospitals, Hematology.
2. Lall Montgomery, M.D., Chairman of the Board of Registry, Muncie, Indiana, Annual Report of Board of Registry.
3. W. D. Davenport, Jr., M.D., Director of Blood Bank, Baptist Hospital, New Orleans, La., Blood Banking.
4. Mr. Morton Reitman, Chief, Laboratory Hazards Section, Headquarters Camp Detrick, Frederick, Maryland, Microbiological Safety. Followed by a film.
5. Harold Jacobs, M.D., Lafayette, La., Cardiac Catheterization.
6. Martin O. Frobisher, M.D., Communicable Disease Center, Chamblee, Ga., Laboratory Diagnosis of Diphtheria.
7. D. L. Tabern, M.D., Abbott Laboratories, Department of Radioactive Pharmaceuticals, Chicago, Illinois. Lecture and Demonstration, The Use of Radioactive Isotopes in Hospital Medical Laboratories.
8. Edward Christian, M.D., Assistant Chief of Medical Service, Veterans Hospital, New Orleans, La., Chromatography.

II. Parasitology Symposium: (See Jan.-Feb. issue of Journal)

III. Parasitology Refresher Course: This will be held on Friday, June 17 at L.S.U. Medical School, conducted by Dr. Marion Hood, Ph.D., Microbiologist, Department of Pathology, Charity Hospital, and Dr. J. Clyde Schwartzwelder, Department of Microbiology, L.S.U. School of Medicine.

Note: Vials of formalized specimens containing parasites will be given registrant at completion of course.
Course limited to 60 students.
Registration Fee—$5.00.

Those desiring to take course please fill in form and mail with check. Checks will be refunded if Registration is closed.

IV. Instrumentation Workshops: The following instrumentation workshops conducted by Rudolph J. Muelling, Jr., M.D., Director of the Instrumentation and Standardization Laboratory, Department of Pathology, L.S.U. School of Medicine, New Orleans, La., will be offered:

1. Calibration (24 persons per session). Each person attending *must* bring 2 hemoglobin pipets and 6 Folin-Wu Sugar tubes.
2. Spectrophotometry (24 persons per session, to work in groups of 4).
3. Ph and Potentiometry with Titration (24 persons per session).
4. Flame Photometry (48 persons per session). Three sessions only to be held.

Time required for each Workshop will be 2 hours. The above sessions will be repeated if Preconvention Registration warrants.

Please fill in form and return if reservation desired.

V. Conferences: The conferences to be part of the Program will not be held concurrently and will not be held as night meetings.

Legislative Conference: Conducted by Miss Virginia Burris, M.T. (ASCP) and Frank Coleman, M.D., Director, Department of Pathology, Mercy Hospital, Des Moines, Iowa.

Educational Conference: Conducted by Mrs. Adabelle N. Thomson, M.T. (ASCP), Miss Verna Rausch, M.T. (ASCP), and Ralph Hartwell, M.D., Director, Department of Pathology, Hotel Dieu, New Orleans, La.

Combined Conference: Conducted by Miss Elizabeth O'Connor, M.T. (ASCP), Chairman, Public Relations, and Mr. Lew Glassner, represented by the following Panelists: Miss Audrey Murphy, M.T. (ASCP), Vocational Guidance and Recruitment Committee; Miss Sylvia Anderson, M.T. (ASCP), Membership; and Mrs. Dallas Johnson, Executive Secretary, National Committee for Careers in Medical Technology.

VI. Papers by ASMT Members: Papers on a variety of topics which should be of interest to all.

Please fill in following forms and return by May 1, 1955:

Patricia M. Sallas, M. T. (ASCP)
Department of Pathology
Charity Hospital
New Orleans, La.

PARASITOLOGY REFRESHER COURSE

Name____________________________

Address____________________________

My check for $5.00________Money Order________is enclosed.

INSTRUMENTATION WORKSHOPS

Check in order of preference the Workshops you wish to attend!

Name____________________________

Address____________________________

☐ Calibration ☐ Ph and Potentiometry with titration

☐ Spectrophotometry ☐ Flame photometry

No Registration Fee required

The Advisory Council (1954-55) will meet on Sunday morning, June 12, at 9:30 A.M. The Advisory Council (1955-56) will meet on Friday, June 17, following the final session of the House of Delegates.

House of Delegates meets on Thursday, June 16, 1 P.M. and Friday, June 17.

FOR THE SISTERS

A reception for the Sisters has been arranged on Sunday at the new modern Mercy Hospital. On Monday evening, June 13, a tea and tour of the New Orleans Charity Hospital.

We feel certain that many of the Sisters will especially enjoy the trip to Carville, the National Leprosarium, conducted by the Sisters of Charity.

Your attention is called to the fact that many of the beautiful churches, including the historic St. Louis Cathedral and Jesuit's Church, are in the vicinity of the Jung Hotel.

HOUSING RESERVATIONS

Convention Headquarters are at the Jung Hotel, 1500 Canal Street, New Orleans, La. Reservations must be made directly with the Hotel.
HOTEL RESERVATIONS: For reservations please fill in the following form and forward to Mr. Rodney Baker, Reservation Dept., Jung Hotel, 1500 Canal Street, New Orleans, La.
PLEASE PRINT

NAME____
Last name First name

ADDRESS______________________________________

CITY__________________ ZONE_____ STATE_________

ADDITIONAL NAMES, IF ANY______________________

____

ARRIVE: DATE_________________HOUR A.M._____P.M._____

PROBABLE DEPARTURE DATE_______________________

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS
23rd Annual Convention
June 12-17, 1955

Please check ( ) accommodations desired. If no room is available at rate requested reservation will be made at next higher rate. Every bedroom has a private bath. Rates subject to 3% Sales Tax.

		$6.50	( )
Room and bath for one	per day	7.00	( )
		8.00	( )
		$9.00	( )
Double bedroom with bath for two	per day	10.00	( )
		11.00	( )
		12.00	( )
		$10.00	( )
Twin bedroom with bath for two	per day	11.00	( )
		12.00	( )
		14.00	( )
Suite (for one or two)	per day	$21.00	
Parlor, bedroom and bath		and up	( )

THE JUNG HOTEL IS COMPLETELY AIR CONDITIONED

OTHER HOUSING: Sisters desiring convent accommodations:
Please contact—Mrs. Maria Hornung
8 Chatham Drive, New Orleans, La.

The Jung Hotel has facilities to house the entire convention. However, if other hotel or motel reservations are desired, please contact:
Miss Marie Louise Dobelman
1929 Robert Street, New Orleans, La.

In order to facilitate local handling of reservations for our colored members, please contact:
Miss Yvonne Stafford
5115 St. Charles Ave., New Orleans, La.

PUBLICITY COMMITTEE PATTER

The Publicity Committee reports active response from most of the States throughout the Country on the sale of seals for the ASMT convention in New Orleans, in June, 1955.

They are available at the following prices:

Individually—1 cent
Lots of 500—$4.50
Lots of 750—$6.75
Lots of 1000—$8.00

As an active member have you been using these seals on your recent mail? If not, you may obtain them on request with remittance to:

Olga S. Blanke
804 Napoleon Avenue
New Orleans 15, La.

SCIENTIFIC EXHIBIT PATTER

Please reserve booth space for your scientific exhibit as early as possible. State Presidents are urged to send in a request form for exhibit space at once.

The backwall of the booth will be medium blue bengaline flameproofed draped material. The background drapes are framed with fancy iron grille work so typically "New Orleans." The side dividers are also made of the black wrought iron grille work. A table is furnished. Covering for the table is to be furnished by the exhibitor.

Please check the following when applying for exhibit space:

1. Size of booth.	All booths are 8 ft. x 6 ft.
2. Counter table.	Needed: Yes: No:
3. Chair.	Needed: Yes: No:
4. Electric outlet. One will be furnished if indicated.	Indicate if needed for display. Additional outlets $7.50
5. Headboard sign.	11" x 28". One or two lines to read:

PLEASE PRINT

Name and address of applicant responsible for exhibit:__________

____

(Signature)

For further particulars or reservation, please write to:

Helen Beros, Chairman
4234 St. Claude Avenue
New Orleans 17, La.

ADVANCE REGISTRATION FORM

You're coming to New Orleans, America's Most Interesting City! No other city in this country is like it. You will want to spend every spare moment seeing and enjoying all the unique features it has to offer. SO WHY WASTE PRECIOUS MOMENTS IN A REGISTRATION LINE!

Fill in the Advance Registration Form and Pick up your Ready Prepared Kit at the table indicated "Advance Registration." Remember to bring your current ASMT membership card as identification.

READ CAREFULLY: Every technologist, member or non-member must pay the registration fee. Guests (family or friends) do not pay this fee. Check in appropriate column each activity you wish to attend. Be sure to check whether member, non-member or guest.

Make checks or money orders payable to the American Society of Medical Technologists and return with the following form properly checked to:

Miss Hazel Newton
1137 Burgundy Street
New Orleans 16, La.

CUT OUT and MAIL NOW

			Technologist		
Sunday, June 12: Sightseeing Tours			Member	Non-member	Guest (No.)
Choice of two tours: 1:00 — 4:30 P.M.	Vieux Carre (old New Orleans) and modern New Orleans tour	$3.75	___	___	___
2:30 — 4:30 P.M.	Vieux Carre Tour (old New Orleans)	$2.00	___	___	___
Tuesday, June 14: 7:30 P.M.	Dinner under the stars at the Court of the Two Sisters, 613 Royal Street—the heart of the Vieux Carre	$5.50	___	___	___
Wednesday, June 15: 12:00 (noon)	Sightseeing Tour of the Harbor aboard the Steamer President	$2.75	___	___	___
Thursday, June 16: 8:00 P.M.	Banquet, Jung Hotel in the "Tulane Room"	$7.50	___	___	___
	Members or non-members not attending the HOUSE OF DELEGATES SESSIONS, if interested, please check.				
1:00 — 5:00 P.M.	Bus tour of Plantation Homes (Oak Alley)*	$2.50	___	___	___
	Admittance cost	1.25	___	___	___
Friday, June 17: 9:00 A.M.	Bus Tour and visit to the National Leprosarium at Carville, La.* (Approximately 5 hours)	$3.00	___	___	___
REGISTRATION FEE		$3.00	___	___	

* **Note:** These trips will be planned if sufficient number interested.

My Check__________Money Order__________for $__________is enclosed.

Advance registration will close June 5th, 1955. After that date you may register on your arrival at the Convention.

PRINT NAME ____________________

AND ADDRESS ____________________

cto

ncer, th
any of y
ours to t
n hospit
ch we h
ce a ca

t hundr
f the Am
Divisi
st medi
by ed
to pati
n prog
s.

te is A
year, 1
e reorg
Society
attac
—far

n the p
upport
r hous